

Novel Computerized Method for Automated Determination of Ventilatory Threshold and Respiratory Compensation Point

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ABSTRACT: The ventilatory threshold (named as VT₁) and the respiratory compensation point (named as VT₂) describe prominent changes of metabolic demand and exercise intensity domains during an incremental exercise test. Methods: A novel computerized method based on the optimization method was developed for automatically determining VT₁ and VT₂ from expired air during a progressive maximal exercise test. A total of 109 peak cycle tests were performed by members of the U.S. astronaut corps (74 males and 35 females). We compared the automatically determined VT₁ and VT₂ values against the visual subjective and independent analyses of 3 trained evaluators. We also characterized VT₁ and VT₂ and the respective absolute and relative work rates and distinguished differences between sexes. Results: The automated compared to the visual subjective values were analyzed for differences with t-test, for agreement with Bland-Altman plots, and for equivalence with a TOST (two one-sided test) approach. The results showed that the automated and visual subjective methods were statistically equivalent, and the proposed approach reliably determined VT₁ and VT₂ values. Females had lower absolute O₂ uptake, work rate, and ventilation, and relative O₂ uptake at VT₁ and VT₂ compared to men ($P \leq 0.04$). VT₁ and VT₂ occurred at a greater relative percentage of their peak VO₂ for females (67% and 88%) compared to males (55% and 74%) (main effect for sex: $P < 0.001$). Overall, VT₁ occurred at 58% of peak VO₂, and VT₂ occurred at 79% of peak VO₂ ($P < 0.0001$). Conclusion: Improvements in determining of VT₁ and VT₂ by automated analysis is time efficient, valid, and comparable to subjective visual analysis, and may provide valuable information in research and clinical practice as well as identifying exercise intensity domains of crewmembers in space.

Keywords: Incremental exercise, noninvasive measurement, ventilatory threshold, respiratory compensation point, automated determination.

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1. INTRODUCTION

Examining the ventilatory profile during incremental exercise to a maximum effort has been used to assess aerobic fitness and monitor and prescribe exercise training in athletes and clinical populations with decades of controversy (Poole et al., 2021, Yeh et al., 1983). Noninvasive measurements of the ventilatory profile are represented commonly as 2 inflection points: the ventilatory threshold (named as VT₁ in this paper) and the respiratory compensation point (named as VT₂ in this paper) (Whipp et al., 1989, Carriere et al., 2019, Cannon et al., 2009, Pallarés et al., 2016, Galán-Rioja et al., 2020). Determining VT₁ and VT₂ during an incremental exercise test should be accompanied by a set of universally agreed upon and explicitly defined quality-control criteria (Galán-Rioja et al., 2020, Poole et al., 2016, Gaskill et al., 2001, Meyer et al., 1996). Several VT methods for detecting VT have traditionally been based on a subjective visual analysis, but this is time consuming, requires 2-3 trained personnel with an independent reviewer and a strict set of criteria to maintain tight quality control (Caiozzo et al., 1982, Dickstein et al., 1990a, Gladden et al., 1985, Shimizu et al., 1991, Yeh et al., 1983).

VT₁ is commonly described as the point at which pulmonary ventilation and carbon dioxide (CO₂) output begin to increase exponentially (Dennis et al., 1992, Whipp et al., 1989). The ventilatory equivalent method has been used to identify VT₁, which is best described as the intensity of activity that causes the first rise in the ventilatory equivalent of oxygen (O₂) without a concurrent rise in the ventilatory equivalent of CO₂ (Powers et al., 1984, Reinhard et al., 1979). Determining VT₁ using the excess CO₂ (ExCO₂) method requires the intensity of exercise that causes an increase from steady state to an excess production of CO₂ (Volkov et al., 1975). Likewise, the V-slope method, also commonly used method, uses points that show an increase in the slope from less than 1 to greater than 1 in the CO₂ production (VCO₂) by O₂ consumption (VO₂) data (Dickstein et al., 1990a, Ekkekakis et al., 2008) and in the minute

ventilation (VE) by VCO₂ data (Beaver et al., 1986) for locating VT₁ and VT₂, respectively. All computerized methods mentioned require finding the point where the slope changes markedly over the entire scattergram. However, 2 regression lines skim the slope of data points from left to right and stop as soon as its criterion is satisfied; thus, the algorithm for searching VT₁ might estimate lower VO₂ compared with the other methods depending on the amount of noise in the data (Ekkkekakis et al., 2008). For the severe exercise intensity domain, VT₂ is considered the second break point at which the partial pressure of arterial CO₂ starts to decline during heavy exercise (Nattie and Li, 2012, Beaver et al., 1986). The 2-line regression model for detecting the slope change in the scattergram also has been used to find VT₂ associated with VE and VCO₂ (Beaver et al., 1986, Davis et al., 1980, Powers et al., 1984, Reinhard et al., 1979, Volkov et al., 1975, Brooks, 1985). However, a disadvantage, inherent to methods that use regression analysis during maximal exercise, is the possibility that the hyperventilation phase (VT₂ starting point) may be partially included in the calculation (Dickstein et al., 1990b).

Exercise countermeasures are the only known way for maintaining muscle mass, strength, and cardiorespiratory fitness in crewmembers during spaceflight. It is important for exercise prescriptions to be optimized to maintain astronauts' fitness to avoid premature physical and cognitive fatigue during performance of high-risk, mission critical tasks. Additionally, more women are now flying into space, yet few studies about sex-specific physiologic differences in the astronaut population have been explored during and after spaceflight. A reliable and automated detection of VT₁ and VT₂ for prescribing exercise domains can help to quickly identify and guide individualized exercise prescriptions for the purpose of maintaining and enhancing crewmembers' performance of submaximal extravehicular activities (EVAs) over long durations. We believe that determining both VT₁ and VT₂ using the proposed novel automated method and their associated absolute and relative work rates can provide valuable information regarding crewmembers' ability to exercise (e.g., buffering capacity, lactate kinetics) on the International Space Station (ISS) and to perform EVAs and lunar exploration. Understanding where VT₁ and VT₂ occur can assist in prescribing exercise intensity above or below those points. Therefore, in this study, we determined the accuracy of our novel automated analysis by comparing the computerized results with subjective visual identification. We also aimed to characterize absolute and relative work rates at VT₁ and VT₂ and to identify if there are any differences in those values between female and male crewmembers.

2. METHODS

2.1. Subjects

This study protocol was reviewed and approved by the NASA Johnson Space Center (JSC)'s Institutional Review Board and in agreement with the Declaration of Helsinki. All participants in the study provided signatures confirming their informed consent. Data were collected from 2013 to 2019. Astronaut participants in these data were active astronauts in flight training at JSC. Exact training status, e.g., amount of time spent in physically training, is not known. A total of 109 peak cycle tests were obtained from a large astronaut database (74 males and 35 females) and used to examine the ventilatory thresholds at VT₁ and VT₂. The data set was separated into groups of female and males for the sex difference study. (Pescatello et al., 2014). See Table 1 for mean participant characteristics and statistical significances for the overall and sex differences.

Table 1. Mean Participant Characteristics and Statistical Significances Between Groups.

Study		Age (year)	Mass (kg)	Height (cm)
Overall		41.1±7.3	76.8±12.4	174.8±9.4
Male vs. Female	Male	41.5±6.8	82.6±9.9	179.1±5.8
	Female	40.3±8.4	64.7±7.2	165.7±9.0
	P-value	0.46	<0.0001	<0.0001

2.2. Test Procedure and Data Collection

Cardiorespiratory fitness (peak VO₂) was determined by a progressive, incremental graded cycle ergometer stress test to volitional exhaustion. Our graded exercise protocol, was developed to measure peak VO₂ and VT at JSC. The protocol has two versions; a nominal protocol and a light protocol conducted on the LODE Excaliber sport cycle ergometer (Lode BV, Groningen, Netherlands). The nominal protocol consisted of a cycling warm-up at 50 W for 3 minutes followed by stepwise increases of 25 W every minute until test termination. The light protocol consists of the same timed wattage increases (i.e. 3 min warm-up then 1-minute increases), but the wattage starts at 45 W and with 15 W increases. Participants were assigned the nominal or light protocol based on body weight. Participants were assigned the light protocol if they weighed less than 65 kilograms. 8 participants fell in this category. We observed

that aerobic response was sufficiently fast to adjust to the 15 or 25 W workload increases within the 1-minute stage time.

Participants were instructed to maintain a cadence of 75 revolutions per minute (RPM) throughout testing. Respiratory gases were sampled per 10-second interval and analyzed using the ParvoMedics TrueOne® 2400 metabolic cart. After a 30-minute warm-up O₂ and CO₂ gas were calibrated using known gases (16% O₂, 4% CO₂) and air flow was calibrated with a 3-L syringe. To ensure accuracy of indirect calorimetry, gas and flow calibration was conducted prior to every exercise test and consisted of ambient and standard gas calibration along with flow meter calibration. Calibration was accepted if new calibration parameters were within +/- 3% of previous values. Additionally, maintenance procedures were followed in accordance with manufacturer guidelines. The rating of perceived exertion (RPE) (Borg scale 6–20) were measured every 2-3 minutes during the exercise test. Heart rate (HR) was determined from 12-lead electrocardiogram recordings throughout the test (CardioSoft CASE®, GE Healthcare, WI, USA). The test was considered to be maximal when at least 4 of 5 following criteria were met: (1) a respiratory exchange ratio (RER) of ≥ 1.10 , (2) a plateau in VO₂ with increasing workloads, (3) workload volitional fatigue (a fall of 10 RPM), (4) exercise peak HR that was within 10 beats of the age-predicted maximal HR ($207 - [0.67 \times \text{age}]$) (Gellish et al., 2007), and (5) RPE at or greater than 19. All participants reached at least 4 of the listed criteria.

In summary, exercise variables in this study were work rates (W), %Wmax (%), HR (beat/min), RER (VO₂:VCO₂), and VE (L/min) at the ventilatory thresholds (VT₁ and VT₂) and peak VO₂ in absolute (L/min) and relative to body weight (mL/kg/min) expressions. The first 3 minutes of warm-up were excluded in data analysis.

2.3. Proposed Computerized Determination of VT Values

Detection of abrupt change in data distribution has been considered as one of the important practical problems arising in various applications (Riedel, 1994). In this study, we used a parametric global optimization method (Lavielle, 2005), which was implemented as a MATLAB function, named *findchangepts* (MATLAB® R2020a, The MathWorks, Inc., Natick, MA, USA). Though the parametric global optimization method identifies a data point change most significantly over the entire scattergram, it could be common during the determination of thresholds to find that some data are indeterminate and inter-method differences are unavoidable in nature (Gaskill et al., 2001). To provide a valid and reliable process, Gaskill et al. recommended to average the combined multiple methods used to identify ventilatory threshold (Gaskill et al., 2001). Thus, we identified VT₁ using the mean of ExCO₂ and V-slope (Figure 1(A)) and detected VT₂ using the mean of the excess minute ventilation (ExVE) method (Kim et al., 2020) and V-slope (Figure 1(B)). Figure 1(C) shows the combined VT₁ and VT₂ corresponded to gas-exchange data: VE/VO₂, ventilatory equivalent for O₂ (blue dots); VE/VCO₂, ventilatory equivalent for CO₂ (red dots); PETO₂, end-tidal pressure of O₂ (green dots); end-tidal pressure of CO₂, PETCO₂ (pink dots). Finally, we identified the work rate associated with VT₁ and VT₂ and compared absolute and relative sex difference.

The ExVE is the method we proposed to identify the intensity of exercise that caused an increase from steady state to excess VE (Kim et al., 2020). Anaerobic exercise triggers a cascade of metabolic reactions in the human body (Beaver et al., 1986). At high intensity of exercise, the high-energy demand triggers a breakdown of glucose and a reduction of pyruvate. These metabolic processes produce lactate faster than the body can metabolize it. Bicarbonate buffers hydrogen H⁺ as a countermeasure when lactate flux increases faster than removal. As exercise intensity increases above this point, VCO₂ increases and systemic pH in the bloodstream decreases as the H⁺ from the bicarbonate is not sufficiently buffered (Myers and Ashley, 1997). This decrease in blood pH triggers carotid bodies, chemoreceptor cells located in the carotid artery, to further increase VE. Therefore, VT₂ can be determined as the point in time where VE increases to compensate for VCO₂ being greater than VO₂, while end tidal CO₂ levels are reduced (Pallarés et al., 2016). We reformulated the typical ExCO₂ concept (e.g., $(\text{VCO}_2^2/\text{VO}_2) - \text{VCO}_2$) (Gaskill et al., 2001, Volkov et al., 1975) to the ExVE form such as $(\text{VE}^2/\text{VCO}_2) - \text{VE}$.

Figure 2 shows the code of the procedure how we generated the input data, how we applied the *findchangepts* function to detect each changepoint location, and how we finally determined VT₁ and VT₂. For example, we specified ‘Statistic’ as ‘std’ because the ‘std’ option detects a significant change that occurs while the standard deviation of input data distribution increases after ventilatory thresholds. Also, we specified ‘MaxNumChanges’ as ‘1’ to return the index of the most significant change point in the scattergrams (see green dashed lines in Figure 1(A) and Figure 1(B)). The algorithm for searching might be more robust and less influenced by unexpected peak noise at the initial or end time range. It has been reported that VT₁ and VT₂ commonly lie at exercise intensities between 50% and 65% of VO₂ and between 75% and 87% of VO₂, respectively (Pallarés et al., 2016, Cerezuola-Espejo et al., 2018, Beaver et al., 1986, Galán-Rioja et al., 2020). Thus, it is recommended to use a specific range of the input signals only, for example, trimming half of the signal length (i.e., from 30th percentile to 80th percentile for VT₁, from 50th percentile to 100th percentile for VT₂).

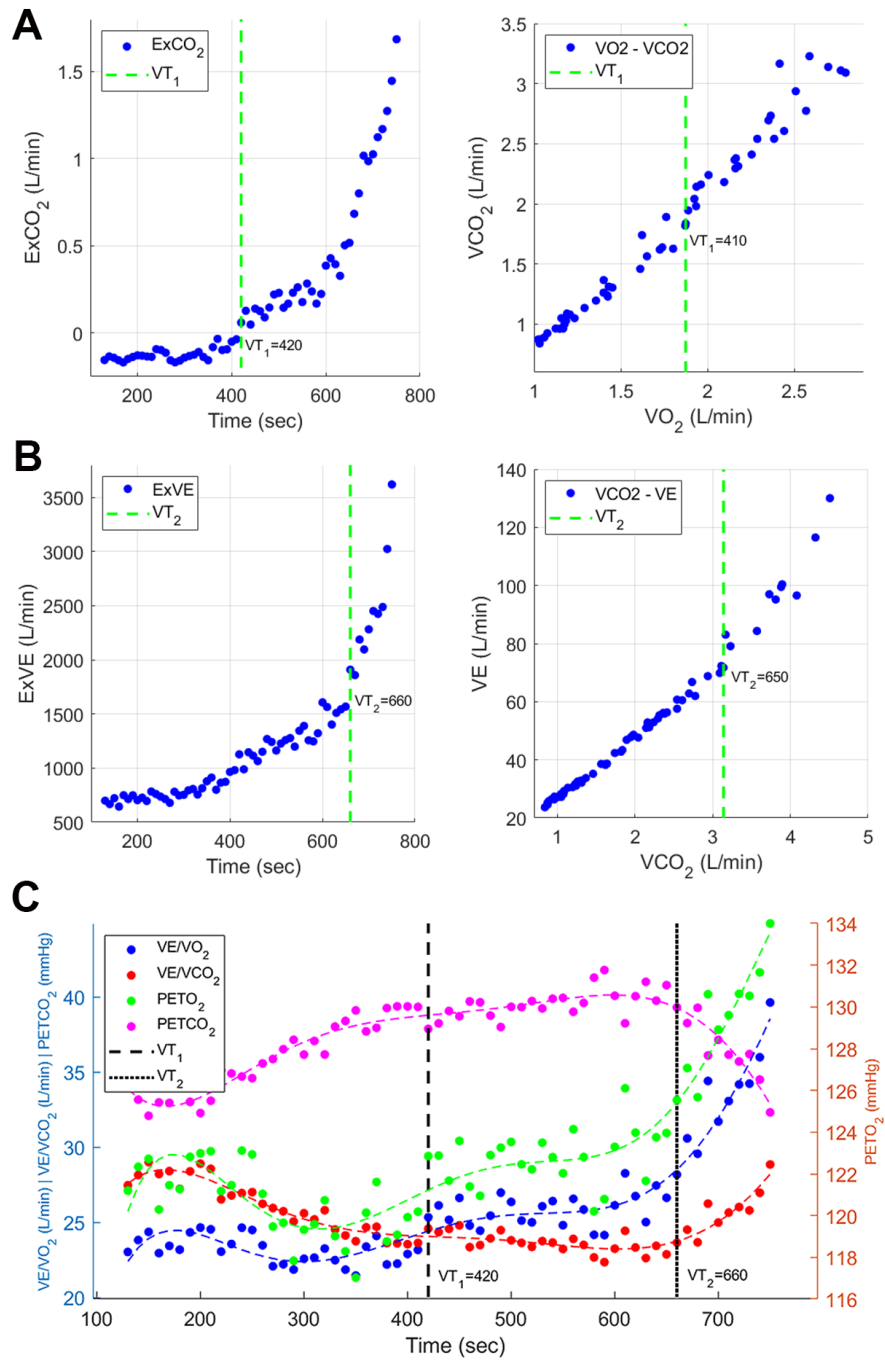


Figure 1. Example of determination of VT₁ and VT₂. (A) left: ExCO₂, right: V-slope for VT₁. (B) left: ExVE, right: V-slope for VT₂. (C) The combined VT₁ and VT₂ corresponded to gas-exchange data: VE/VO₂, ventilatory equivalent for O₂ (blue dots); VE/VCO₂, ventilatory equivalent for CO₂ (red dots); PETO₂, end-tidal pressure of O₂ (green dots); end-tidal pressure of CO₂, PETCO₂ (pink dots).

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Input:
VCO2: a vector with N elements (n×1) %% Rate of CO2 production Volume of CO2/min
VO2 : a vector with N elements (n×1) %% Rate of O2 Consumption Volume of O2/min
VE : a vector with N elements (n×1) %% Minute ventilation Volume of expired air/min
Process:
1 ExCO2 = ((VCO2 .* VCO2) ./ VO2) - VCO2 %% Excess carbon dioxide method (VT1)
2 ipt_ExCO2= findchangepts(ExCO2, 'Statistic', 'std', 'MaxNumChanges', 1)
3 Vslope1 = [VO2 VCO2]' %% V-slope (VT1)
4 ipt_Vslope1 = findchangepts(Vslope1, 'Statistic', 'std', 'MaxNumChanges', 1)
5 ExVE = ((VE .* VE) ./ VCO2) - VE %% Excess minute ventilation method (VT2)
6 ipt_ExVE = findchangepts(ExVE, 'Statistic', 'std', 'MaxNumChanges', 1)
7 Vslope2 = [VCO2 VE]' %% V-slope (VT2)
8 ipt_Vslope2 = findchangepts(Vslope2, 'Statistic', 'std', 'MaxNumChanges', 1)
9 vt1 = round((ipt_ExCO2 + ipt_Vslope1) / 2)
10 vt2 = round((ipt_ExVE + ipt_Vslope2) / 2)
Output:
ipt_ : Changepoint locations
vt1 : Ventilation threshold (VT1) location
vt2 : Respiratory compensation point (VT2) location

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Figure 2. Code for the suggested approach.

2.4. Visually Evaluated VT Values

For the validation study, 3 trained evaluators independently and randomly evaluated the graphs of the data to determine VT₁ and VT₂ values. For each determination, graphs were visually evaluated for the assessment of change in data distribution. Specifically, for VT₁, evaluators assessed the intensity of activity that causes the first sustained rise in the VE/VO₂ without a concurrent rise in the ventilatory VE/VCO₂ (Figure 3(A)). The rise in VE/VO₂ is in concurrence with RER reaching 1.0. For VT₂, evaluators assessed increase in both the VE/VO₂ and VE/VCO₂ (Figure 3(B)). This rise in VE/VO₂ and VE/VCO₂ is in concurrence with a decrease in PetCO₂ (5). A detailed protocol to maintain tight quality control over determination of VT₁ and VT₂ values was developed and included the following rules: 1) if, after concurrently viewing all graphs, an evaluator still thought that the VT value was indeterminate, then data for that subject were rejected. If the evaluators thought that data were usable, they then chose what they thought to be the most representative value. 2) The values determined by the 3 independent investigators were then compared by a fourth independent investigator. If the values determined by the evaluators were within 1 exercise stage (50 W or less than 15 %), then values for the 3 investigators were averaged. 3) If values were within 15 % of either of the initial investigators, then the VT values were averaged. Comparison values greater than 15% were removed from the analysis. 4) The appearance time of VT needed to be after the 75 W (after 4 minutes) warm up of the exercise test or the data were rejected and were considered to be indeterminate. The data reported for the visual identification method include only those participants whose data met all the criteria. Evaluators rejected 57 for VT₁ and 28 for VT₂ based on our criteria. For the accurate comparison between the visual analysis and the automated analysis, we only used the paired matches.

2.5. Statistical Analysis

Independent t-tests were used when examining subject characteristics and sex comparison of exercise variables for VO₂, W, HR, RER, and VE values at VT₁ and VT₂.

The mean differences of the subjective analysis and the automated analysis were examined using independent t-test to test for differences between measures. The Bland-Altman analysis (Giavarina, 2015) assessed the limits of agreement between VT₁ and VT₂. Formal equivalence testing was conducted with the TOST approach with predefined equivalence bounds of ±25 (Schuirmann, 1987). The data were also analyzed by the intraclass correlation coefficients (ICC) to examine the relationships between the subjective analysis and the automated analysis.

For each subject, absolute VO₂, relative VO₂, W, HR, RER, and VE were determined at VT₁ (average of V-slope for VT₁ and ExCO₂ methods), VT₂ (average of V-slope for VT₂ and ExVE methods), and peak, a 2-way factorial analysis of variance (ANOVA) was then used to examine main effects and interactions (VT × Group) for the sex difference study. If significance was found, the appropriate Holm-Sidak multiple comparison post hoc test was performed.

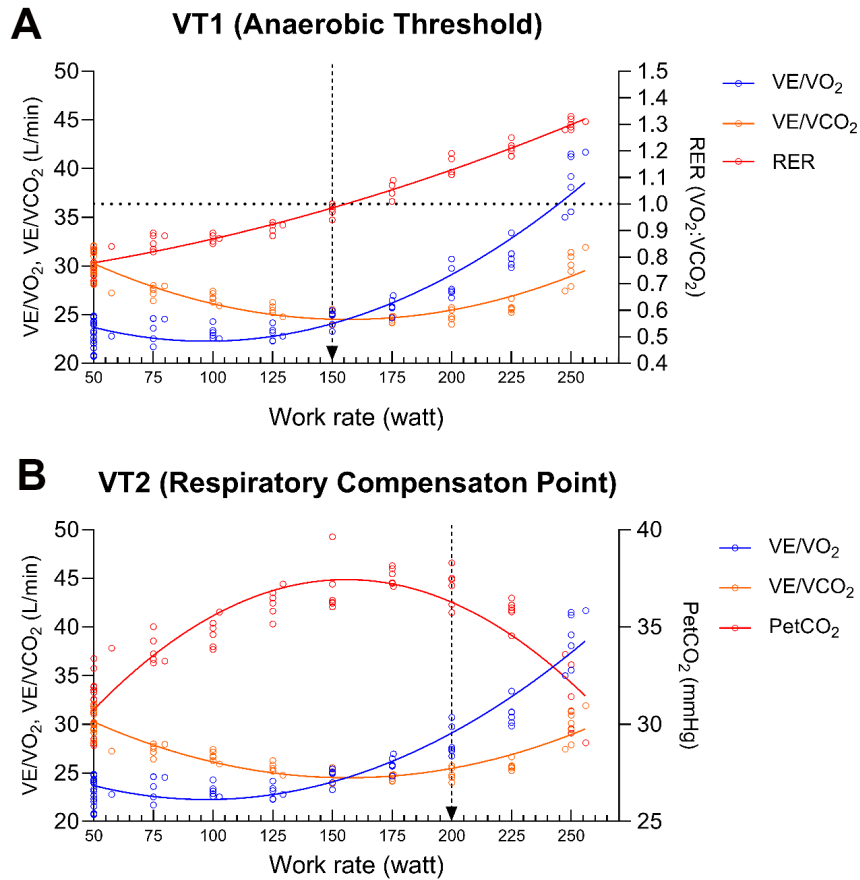


Figure 3. Visual determination of VT_1 (A) and VT_2 (B). The arrows designate the choice of VT_1 and VT_2 in these sample graphs from one subject.

Data were analyzed and figures generated using MATLAB R2020a (The MathWorks, Inc., Natick, MA, USA) and GraphPad Prism (Version 8.4.3, La Jolla, CA, USA) with statistical significance set at $P < 0.05$. Equivalence testing was conducted with the TOSTER package in the R statistical software (R: A Language and Environment for Statistical Computing, R Core Team, R Foundation for Statistical Computing, Vienna Austria, 2019, <https://www.R-project.org>). All data are reported as mean \pm SD.

3. RESULTS

3.1. Subject Characteristics and Sex Comparison of Exercise Variables at VT_1 and VT_2

Males and females were matched for age but different for mass and height (Table 1). Mean exercise variables for the overall subjects and sex difference are reported in Table 2. At peak, VT_1 , and VT_2 , females had lower absolute O_2 uptake, W, and ventilation and relative O_2 uptake compared to men ($P \leq 0.04$). HRs and RERs were not different at peak exercise for either group. However, HRs and RERs were lower in the male group compared to the female group at VT_1 and VT_2 ($P \leq 0.02$).

3.2. Relative Exercise Intensities at VT_1 and VT_2

Exercise intensities at VT_1 and VT_2 relative to peak VO_2 and peak W for the combined analysis in all subjects and sex differences are reported in Figure 4. In the combined analysis, VT_1 occurred at 58% of peak VO_2 and VT_2 occurred at 79% of peak VO_2 ($P < 0.0001$). Work rates at VT_1 and VT_2 were 50% and 69% of peak W ($P < 0.0001$). For the sex difference comparison, VT_1 and VT_2 occurred at a greater relative percentage of their peak VO_2 for females (67% and 88%) compared to males (55% and 74%), main effect for group; $P < 0.001$. However, no differences were found for group expressed as a relative percentage of peak W. Both sexes had VT_1 at 50% and VT_2 at 70% of peak W ($P \geq 0.40$).

Table 2. Mean Values at peak, VT₁, and VT₂.

Study		VO ₂ (L/min)	VO ₂ (mL/kg/min)	Work Rate (W)	HR (beat/min)	RER (VO ₂ :VCO ₂)	VE (L/min)	
Values at peak	Overall	3.3±0.9	43.2±7.7	303.3±76.4	177±10	1.28±0.08	140.0±37.0	
	Male vs. Female	Males	3.8±0.6	45.8±7.1	339.9±48.4	178±8	1.29±0.07	156.4±31.1
		Females	2.4±0.4	37.8±5.8	225.9±66.5	177±13	1.28±0.09	105.3±21.2
		<i>P</i> -value	<0.0001	<0.0001	<0.0001	0.74	0.48	<0.0001
Values at VT ₁	Overall	1.9±0.5	25.2±4.5	156.2±43.9	137±15	0.99±0.06	52.0±11.3	
	Male vs. Female	Males	2.1±0.4	25.7±4.8	172.3±39.8	134±14	1.00±0.05	55.6±10.4
		Females	1.6±0.3	24.0±3.4	120.2±28.9	144±15	0.97±0.08	44.5±9.4
		<i>P</i> -value	<0.0001	0.04	<0.0001	<0.01	0.02	<0.0001
Values at VT ₂	Overall	2.6±0.6	33.3±6.3	213.8±53.2	155±17	1.10±0.06	77.0±15.3	
	Male vs. Female	Males	2.8±0.5	34.3±6.3	234.3±45.3	151±17	1.10±0.06	82.2±13.7
		Females	2.1±0.4	31.3±6.0	167.7±39.3	162±13	1.10±0.08	65.8±12.1
		<i>P</i> -value	<0.0001	0.01	<0.0001	<0.01	0.88	<0.0001

*VO₂ = volume of oxygen; HR = heart rate; RER = respiratory exchange ratio; VE =ventilation; VT = ventilatory threshold

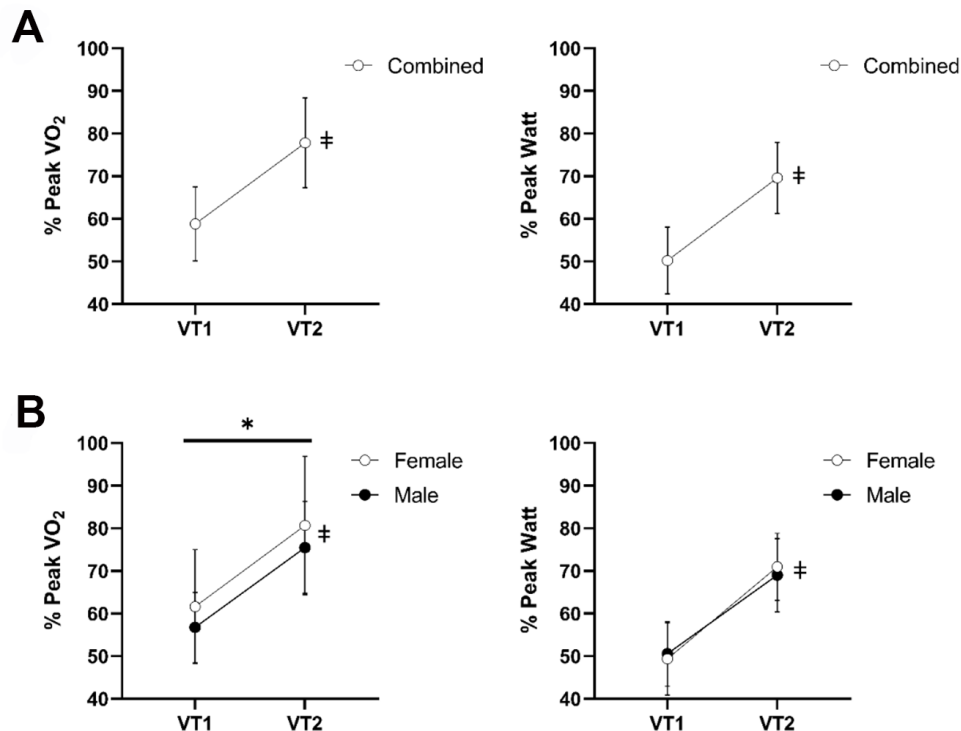


Figure 4. Exercise intensities at VT₁ and VT₂ relative to peak VO₂ and peak W for the combined analysis (A) and sex differences (B). *Indicates group main effect differences *P*<0.001. ‡ indicates significant main effect difference between VT₁ and VT₂ *P*<0.0001. Data reported as means ± SD.

3.3. Comparison between Visual and Automated

Independent t-tests for the comparison between the subjective analysis and the automated analysis found no difference for VT₁ ($P>0.05$) and VT₂ ($P>0.05$). Figure 5 illustrates the Bland-Altman plot. Bias for VT₁ was 7.08 ± 12.2 W with the 95% Limits of Agreement from -16.99 to 31.06 W. Bias for VT₂ was -2.2 ± 12.7 W with the 95% Limits of Agreement from -27.0 to 22.7 W. For VT₁ and VT₂ combined, the equivalence test was significant, $t(132) = -9.389$, $P<0.0001$, given equivalence bounds of -25 and 25 (on a raw scale) and an alpha of 0.05. For VT₁ and VT₂ individually, the equivalence tests also were significant, $t(51) = -4.513$, $P<0.0001$ and $t(80) = 5.812$, $P<0.0001$, respectively, given equivalence bounds of -25 and 25 (on a raw scale) and an alpha of 0.05. Equivalence testing showed that the automated and visual measures were statistically equivalent ($P<0.0001$) in all ways, each VT₁ or VT₂ and combined. Finally, a high degree of reliability was found between the subjective analysis and the automated analysis. The ICC between the subjective analysis and the automated analysis were 0.821 for VT₁ and 0.830 for VT₂ with a 95% confidence interval.

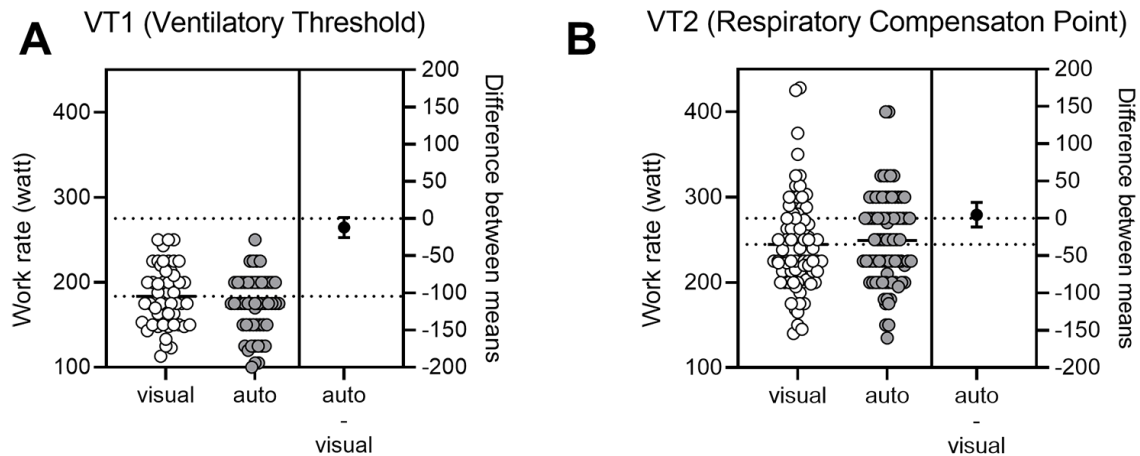


Figure 5. Bland-Altman Analysis between visual and automated method. (A) VT₁, (B) VT₂.

4. DISCUSSION

The advantages of a computerized method include faster, objective, and automated data analysis as well as improvements in reproducibility and repeatability. The aim of this study was to provide a novel, reliable, and computerized method for automatically identifying VT₁ and VT₂. We demonstrated that our method was able to determine ventilatory thresholds comparable to the visual analysis accomplished by 3 trained evaluators. We also determined the associated work rates expressed as absolute and relative submaximal VO₂ and W and reported that sex differences exist for VT₁ and VT₂.

Others have compared various computerized methods for determining VT₁ and demonstrated that regression-based methods provide considerably different results (Ekkekakis et al., 2008). For example, VT₁ values detected by using 2 regression lines were significantly lower with weaker correlations compared to other computerized methods. Additionally, Pearson correlation coefficients in VO₂ (L/min) between ExCO₂ and V-slope for VT₁ were 0.517 and 0.526 (i.e., a moderate positive relationship) in each sample group. In this study, Pearson correlation coefficient in VO₂ (L/min) detected by ExCO₂ and V-slope for VT₁ using the parametric global optimization method was 0.81 (i.e., a strong positive linear relationship), which is a stronger correlation coefficient compared to using 2 regression lines (Ekkekakis et al., 2008). We also found that the strong positive relationship for Pearson correlation coefficient between computerized methods for determining VT₂ (ExVE and V-slope for VT₂) between ExVE and V-slope for VT₂ means that the parametric global optimization method. In support of these, we found no difference between the subjective visual method by 3 trained evaluators and our automated method. Our novel and automated protocol may increase the methodological consistency in both research and clinical practice.

It has been reported that maximal aerobic capacity is associated with VT₁ (59% to 65%) and VT₂ (84% to 87%) and that maximal lactate steady state corresponds to VT₂ (Pallarés et al., 2016, Cerezuela-Espejo et al., 2018). We found that when comparing to peak work rate, VT₁ and VT₂ were associated with 50% and 70% of peak W, respectively. These differences may be because of age and fitness of our study participants' steady state vs. progressive exercise test. When we examined the ventilatory points in relation to peak VO₂, the female group had greater relative VO₂ associated with VT₁ (67%) and VT₂ (88%). Beaver et al. reported that VT₁ occurred at 55% of peak VO₂ and

VT₂ occurred at 75% of peak VO₂ (Beaver et al., 1986) and a recent meta-analysis reported that VT₁ occurs at 50% to 60% of peak VO₂ (Galán-Rioja et al., 2020). These values are similar to ours as we report the whole sample was at 58% and 79% of peakVO₂ for VT₁ and VT₂, respectively. As noted earlier, these differences in VT₂ for the female group may be because of sensitivity of the carotid body ventilatory drive caused by body temperature, blood osmolarity, pH, K⁺, H⁺ buffering by bicarbonate, and the change in partial pressure of O₂ (Galán-Rioja et al., 2020).

It has been reported that microgravity affects females and males differently (Mark et al., 2014). It is important to better understand these sex differences as the female representation in the astronaut corps is increasing, meaning more women will be eligible to fly in space than ever before. Under the microgravity environment, one of the sex-specific differences in exercise response is orthostatic intolerance caused by plasma volume loss and cardiovascular adjustments (Goel et al., 2014). Females generally have smaller body size, lower absolute and relative aerobic fitness, and are weaker in upper and lower body strength, which have implications for risk of fatigue and injury from muscle strains during EVA and emergency egress (Harm et al., 2001). Thus, understanding exercise countermeasures and the adaptations between sexes is of high relevance for the astronaut population. Our data is similar to others that report sex differences in gas exchange threshold for VT₁. In this study, we also showed that VT₂ differences occurred between males and females. This may be because of differences in breathing adjustments to chemosensitivity, thermoregulation, and menstrual cycle hormones (Beaver et al., 1986, Kilbride et al., 2003, Hayashi et al., 2012).

Others have suggested using exercise work rates above and below the ventilatory breakpoints for VT₁ and VT₂ for the prescription of exercise training to define exercise domains such as moderate, heavy-and severe exercise intensity domains (Nattie and Li, 2012, Beaver et al., 1986). This gives an individualized approach to prescribe exercise specific to the metabolic demands. Previous exercise training countermeasures during spaceflight or analogues have used a relative percentage of peak VO₂ (e.g., continuous cycle exercise for 30 minutes at 75% of peak VO₂ and interval treadmill sessions of 30 seconds to 4 minutes at nearly maximal intensity) (Moore Jr et al., 2014, Ploutz-Snyder et al., 2018). However, the responses to these exercise prescriptions still have high variability for maintaining fitness. For example, Moore et al. reported that astronauts who have higher initial aerobic capacities are more prone to loss of cardiorespiratory fitness; however, the reason is unknown and may be because of the frequency, intensity, time, and progression of the exercise prescription (Moore Jr et al., 2014). Our data suggest that sex should be considered when prescribing exercise countermeasures and the prescriptions could be further individualized by prescribing based on VT₁, VT₂, and peak VO₂.

Notably, we acknowledge a limitation with our study. This includes not obtaining arterial lactate samples and blood gasses to confirm the metabolic and ventilation breakpoints. Further validation should include these measurements.

5. CONCLUSION

In summary, the new automated method has been shown to identify inflection points in each of the variables used to reliably determine VT₁ and VT₂. Furthermore, we show that sex influences the VT₁ and VT₂ in members of the U.S. astronaut corps. Detection of both VT₁ and VT₂ and their associated absolute and relative work rates may provide valuable information regarding crewmembers' ability to exercise on the ISS and to perform EVAs and lunar exploration. Lastly, accurately tracking fitness pre-, in-, and post-flight is of importance for guidance on the efficacy of exercise training prescriptions as countermeasures.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REFERENCES

- BEAVER, W. L., WASSERMAN, K. & WHIPP, B. J. 1986. A new method for detecting anaerobic threshold by gas exchange. *Journal of Applied Physiology*, 60, 2020-2027.
- BROOKS, G. A. 1985. Anaerobic threshold: review of the concept and directions for future research. *Medicine & Science in Sports & Exercise*, 17, 22-34.
- CAIOZZO, V. J., DAVIS, J. A., ELLIS, J. F., AZUS, J. L., VANDAGRIFF, R., PRIETTO, C. & MCMASTER, W. 1982. A comparison of gas exchange indices used to detect the anaerobic threshold. *Journal of Applied Physiology*, 53, 1184-1189.

- CANNON, D. T., KOLKHORST, F. W. & BUONO, M. J. 2009. On the determination of ventilatory threshold and respiratory compensation point via respiratory frequency. *International Journal of Sports Medicine*, 30, 157-162.
- CARRIERE, C., CORRÀ, U., PIEPOLI, M., BONOMI, A., MERLO, M., BARBIERI, S., SALVIONI, E., BINNO, S., MAPELLI, M. & RIGHINI, F. 2019. Anaerobic threshold and respiratory compensation point identification during cardiopulmonary exercise tests in chronic heart failure. *Chest*, 156, 338-347.
- CEREZUELA-ESPEJO, V., COUREL-IBÁÑEZ, J., MORÁN-NAVARRO, R., MARTÍNEZ-CAVA, A., & PALLARÉS, J. G. 2018. The relationship between lactate and ventilatory thresholds in runners: validity and reliability of exercise test performance parameters. *Frontiers in Physiology*, 9, 1-10.
- DAVIS, J. A., WHIPP, B. J. & WASSERMAN, K. 1980. The relation of ventilation to metabolic rate during moderate exercise in man. *European Journal of Applied Physiology and Occupational Physiology*, 44, 97-108.
- DENNIS, S. C., NOAKES, T. D. & BOSCH, A. N. 1992. Ventilation and blood lactate increase exponentially during incremental exercise. *Journal of Sports Sciences*, 10, 437-449.
- DICKSTEIN, K., BARVIK, S., AARSLAND, T., SNAPINN, S. & KARLSSON, J. 1990a. A comparison of methodologies in detection of the anaerobic threshold. *Circulation*, 81, II38-46.
- DICKSTEIN, K., BARVIK, S., AARSLAND, T., SNAPINN, S. & MILLERHAGEN, J. 1990b. Validation of a computerized technique for detection of the gas exchange anaerobic threshold in cardiac disease. *The American Journal of Cardiology*, 66, 1363-1367.
- EKKEKAKIS, P., LIND, E., HALL, E. E. & PETRUZZELLO, S. J. 2008. Do regression-based computer algorithms for determining the ventilatory threshold agree? *Journal of Sports Sciences*, 26, 967-976.
- GALÁN-RIOJA, M. Á., GONZÁLEZ-MOHÍNO, F., POOLE, D. C. & GONZÁLEZ-RAVÉ, J. M. 2020. Relative Proximity of Critical Power and Metabolic/Ventilatory Thresholds: Systematic Review and Meta-Analysis. *Sports Medicine*, 1-13.
- GASKILL, S. E., RUBY, B. C., WALKER, A. J., SANCHEZ, O. A., SERFASS, R. C. & LEON, A. S. 2001. Validity and reliability of combining three methods to determine ventilatory threshold. *Medicine & Science in Sports & Exercise*, 33, 1841-1848.
- GELLISH, R. L., GOSLIN, B. R., OLSON, R. E., MCDONALD, A., RUSSI, G. D. & MOUDGIL, V. K. 2007. Longitudinal modeling of the relationship between age and maximal heart rate. *Medicine & Science in Sports & Exercise*, 39, 822-829.
- GIAVARINA, D. 2015. Understanding bland altman analysis. *Biochemia Medica*, 25, 141-151.
- GLADDEN, L. B., YATES, J., STREML, R. W. & STAMFORD, B. A. 1985. Gas exchange and lactate anaerobic thresholds: inter-and intraevaluator agreement. *Journal of Applied Physiology*, 58, 2082-2089.
- GOEL, N., BALE, T. L., EPPERSON, C. N., KORNSTEIN, S. G., LEON, G. R., PALINKAS, L. A., STUSTER, J. W. & DINGES, D. F. 2014. Effects of sex and gender on adaptation to space: behavioral health. *Journal of Women's Health*, 23, 975-986.
- HARM, D. L., JENNINGS, R. T., MECK, J. V., POWELL, M. R., PUTCHA, L., SAMS, C. P., SCHNEIDER, S. M., SHACKELFORD, L. C., SMITH, S. M. & WHITSON, P. A. 2001. Invited review: gender issues related to spaceflight: a NASA perspective. *Journal of Applied Physiology*, 91, 2374-2383.
- HAYASHI, K., KAWASHIMA, T. & SUZUKI, Y. 2012. Effect of menstrual cycle phase on the ventilatory response to rising body temperature during exercise. *Journal of Applied Physiology*, 113, 237-245.
- KILBRIDE, E., MCLOUGHLIN, P., GALLAGHER, C. G. & HARTY, H. R. 2003. Do gender differences exist in the ventilatory response to progressive exercise in males and females of average fitness? *European Journal of Applied Physiology*, 89, 595-602.
- KIM, K. J., BUXTON, R., CROWELL, J., DOWNS, M. & ABERCROMBY, A. 2020. An Automated Excess Minute Ventilation Method To Detect The Respiratory Compensation. *Medicine & Science in Sports & Exercise*, 52, 37-39.
- LAVIELLE, M. 2005. Using penalized contrasts for the change-point problem. *Signal Processing*, 85, 1501-1510.
- MARK, S., SCOTT, G. B., DONOVIEL, D. B., LEVETON, L. B., MAHONEY, E., CHARLES, J. B. & SIEGEL, B. 2014. The impact of sex and gender on adaptation to space: executive summary. *Journal of Women's Health*, 23, 941-947.
- MEYER, K., HAJRIC, R., WESTBROOK, S., SAMEK, L., LEHMANN, M., SCHWAIBOLD, M., BETZ, P. & ROSKAMM, H. 1996. Ventilatory and lactate threshold determinations in healthy normals and cardiac patients: methodological problems. *European Journal of Applied Physiology and Occupational Physiology*, 72, 387-393.

- MOORE JR, A. D., DOWNS, M. E., LEE, S. M., FEIVESON, A. H., KNUDSEN, P. & PLOUTZ-SNYDER, L. 2014. Peak exercise oxygen uptake during and following long-duration spaceflight. *Journal of Applied Physiology*, 117, 231-238.
- MYERS, J. & ASHLEY, E. 1997. Dangerous curves: a perspective on exercise, lactate, and the anaerobic threshold. *Chest*, 111, 787-795.
- NATTIE, E. & LI, A. 2012. Central chemoreceptors: locations and functions. *Comprehensive Physiology*, 2, 221-254.
- PALLARÉS, J. G., MORÁN-NAVARRO, R., ORTEGA, J. F., FERNÁNDEZ-ELÍAS, V. E. & MORA-RODRIGUEZ, R. 2016. Validity and reliability of ventilatory and blood lactate thresholds in well-trained cyclists. *PLoS one*, 11, 1-16.
- PESCATELLO, L. S., RIEBE, D. & THOMPSON, P. D. 2014. *ACSM's guidelines for exercise testing and prescription*, Lippincott Williams & Wilkins.
- PLOUTZ-SNYDER, L. L., DOWNS, M., GOETCHIUS, E., CROWELL, B., ENGLISH, K. L., PLOUTZ-SNYDER, R., RYDER, J. W., DILLON, E. L., SHEFFIELD-MOORE, M. & SCOTT, J. M. 2018. Exercise training mitigates multisystem deconditioning during bed rest. *Medicine and Science in Sports and Exercise*, 50, 1920-1928.
- POOLE, D. C., BURNLEY, M., VANHATALO, A., ROSSITER, H. B. & JONES, A. M. 2016. Critical power: an important fatigue threshold in exercise physiology. *Medicine and Science in Sports and Exercise*, 48, 2320-2334.
- POOLE, D. C., ROSSITER, H. B., BROOKS, G. A. & GLADDEN, L. B. 2021. The anaerobic threshold: 50+ years of controversy. *The Journal of Physiology*, 599, 737-767.
- POWERS, S. K., DODD, S. & GARNER, R. 1984. Precision of ventilatory and gas exchange alterations as a predictor of the anaerobic threshold. *European Journal of Applied Physiology and Occupational Physiology*, 52, 173-177.
- REINHARD, U., MÜLLER, P. & SCHMÜLLING, R.-M. 1979. Determination of anaerobic threshold by the ventilation equivalent in normal individuals. *Respiration*, 38, 36-42.
- RIEDEL, K. S. 1994. Detection of abrupt changes: theory and application. Taylor & Francis.
- SCHUIRMANN, D. J. 1987. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *Journal of Pharmacokinetics and Biopharmaceutics*, 15, 657-680.
- SHIMIZU, M., MYERS, J., BUCHANAN, N., WALSH, D., KRAEMER, M., MCAULEY, P. & FROELICHER, V. F. 1991. The ventilatory threshold: method, protocol, and evaluator agreement. *American Heart Journal*, 122, 509-516.
- VOLKOV, N. I., SHIRKOVETS, E. A. & BORILKEVICH, V. E. 1975. Assessment of aerobic and anaerobic capacity of athletes in treadmill running tests. *European Journal of Applied Physiology and Occupational Physiology*, 34, 121-130.
- WHIPP, B. J., DAVIS, J. A. & WASSERMAN, K. 1989. Ventilatory control of the isocapnic buffering region in rapidly-incremental exercise. *Respiration Physiology*, 76, 357-367.
- YEH, M. P., GARDNER, R. M., ADAMS, T., YANOWITZ, F. & CRAPO, R. 1983. "Anaerobic threshold": problems of determination and validation. *Journal of Applied Physiology*, 55, 1178-1186.