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# Day-to-Day Variation of Cardiopulmonary Variables Obtained During an Incremental Cycling Test to Volitional Exhaustion

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<sup>1</sup>Biometrics Laboratory, School of Physical Education and Sports, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; <sup>2</sup>Postgraduate in Physical Education, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; <sup>3</sup>Brain Mapping and Sensory Motor Integration, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

#### ABSTRACT

Paulucio D, Nogueira F, Velasgues B, Ribeiro P, Pompeu F. Dayto-Day Variation of Cardiopulmonary Variables Obtained During an Incremental Cycling Test to Volitional Exhaustion. JEPonline 2015; 18(3):81-90. The purpose of this study was to analyze the reliability of measurements of oxygen consumption (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>) and expired ventilation ( $V_E$ ) by the Vista Mini-CPX® Vacumed® system. The protocol consisted of two identical, continuous efforts on an electromagnetically braked training ergometer. Sixteen trained male cyclists (32  $\pm$  6 yrs, 69.5  $\pm$  6.1 kg) participated in the study. There was no significant difference (P>0.05) between test and retest measurements on the device at different intensities. Measurements of test and retest of each respiratory variable were significantly correlated, with  $r^2 = 0.95$ , 0.90, and 0.95 and intraclass correlation coefficient 0.98, 0.96, and 0.98 VO<sub>2</sub>, VCO<sub>2</sub>, and V<sub>F</sub>, respectively. The Vista Mini-CPX®, Vacumed® system can be considered a reliable tool to measure and assess the cardiorespiratory performance of subjects in a laboratory or research setting.

**Key Words:** Reproducibility, Oxygen Consumption, Carbon Dioxide Production, Indirect Calorimetry

## INTRODUCTION

Measurements of oxygen consumption (VO<sub>2</sub>), the volume of carbon dioxide produced (VCO<sub>2</sub>), and expired (i.e., minute) ventilation (V<sub>E</sub>) performed by open-circuit indirect calorimetry are extremely important for exercise physiologists and sports medicine professionals (11,13,22,26). Such results contribute to the evaluation of metabolic disorders, the characterization of metabolic demand during training and sports competitions, the indirect estimation of energy substrates during rest and exercise, and the measurement of changes in VO<sub>2</sub> in response to specific interventions (20,28,33).

The literature describes a wide variety of instruments for measuring respiratory gases (particularly,  $VO_2$ ,  $VCO_2$ , and  $V_E$ ) in exercise physiology (5,6,8,24,30). These instruments can be found in stationary as well as portable systems that are absolutely imperative to carrying out scientific and clinical research. Hence, it is also important that the instruments produce reliable data. This is why reliability studies are relatively common in checking the reproducibility of the measures at rest and during exercise (23,26). In instances where the reliability facts for a given system are not available (7), researchers must determine if the system is reliable.

One such equipment mentioned in the literature is the Vista Mini-CPX<sup>®</sup> system (Vacumed<sup>®</sup> Ventura, CA, USA). According to the manufacturer, this stationary system provides excellent reliability, allowing its use in scientific studies. The use of this equipment to investigate different indices of ventilatory efficiency has been reported in the literature (9). However, to confirm these findings, studies of reliability of the measurements are needed. The purpose of this study was to assess the reliability of the measurements of VO<sub>2</sub>, VCO<sub>2</sub>, and V<sub>E</sub> using the Vista Mini-CPX with trained cyclists during a progressive exercise load and a maximum exercise test protocol on an electromagnetically braked training ergometer.

#### METHODS

#### Subjects

This study was divided into two identical trials (M1 and M2), comprising 16 male volunteers  $(32 \pm 6 \text{ yrs}, 69.5 \pm 6.1 \text{ kg})$ . All subjects were non-smokers who were apparently healthy and road-trained cyclists. They were requested to: (a) maintain a mixed diet during the 48 hrs prior to the measurement sessions; (b) refrain from extenuating physical activities (>5 METs) and alcohol ingestion for 24 hrs before the test; and (c) refrain from food and caffeine for 3 hrs before the test. Each subject was informed of the risk associated with the test. An informed consent statement was read and signed by each subject. The experimental protocol was approved by the local ethics committee for experiments with humans.

#### Protocols

The rear wheel of a racing bike was coupled to an electromagnetically braked training device (CompuTrainer®, Lab 3D, RacerMate, Seattle, USA). Each subject performed the test on his own bike, but the same rear tire and wheel was used for all tests. After seated on the bike without pedaling for 6 min, baseline values were obtained. The subjects performed a warm-up for 4 min with 30-W load and, then, began the scaled phase with a load of 100 W incrementing by 30 W every 3 min until the maximum voluntary level was attained and fatigue occurred. The subjects chose the preferred cadence between values of 1.17 and 1.5 Hertz, which is 70 to 90 rev-min<sup>-1</sup>.

Maximum effort was defined as meeting at least three of the following criteria (17): (a) plateau in which the VO<sub>2</sub> increase was  $\leq 150 \text{ mL} \cdot \text{min}^{-1}$  or 2 mL·kg<sup>-1</sup>·min<sup>-1</sup>; (b) respiratory exchange ratio of

≥1.15; (c) 90% of maximum heart rate according to age (220 - age); (d) blood lactate concentration ≥8 mmol·L<sup>-1</sup>; and (e) voluntary fatigue, that is, the subjects' inability to keep the pre-established rhythm. The VO<sub>2</sub> max was determined as the average value generated in the last 30 sec at the end of the test. The interval between M1 (test) and M2 (retest) was at least 48 hrs and no more than 7 days.

The metabolic analysis was conducted by open-circuit indirect calorimetry (Vista Mini-CPX<sup>®</sup>, Vacumed<sup>®</sup>, Ventura, CA, USA). Each subject's V<sub>E</sub> was measured across a turbine flow sensor with dynamic resistance <0.5 cm H<sub>2</sub>O·L<sup>-1</sup>·s<sup>-1</sup> (12 L·sec<sup>-1</sup>) and an error of ± 2% in accuracy (2). The volume fractions of expired oxygen and carbon dioxide were measured, respectively, using a cold fuel cell system and an infrared sensor with an error of ±3% according to the manufacturer's specifications (2). The subjects used a silicone mask (V-Mask<sup>®</sup>, Hans Rudolph Inc<sup>®</sup>,Kansas City, MO, USA) coupled to a bidirectional turbine flow of gases ("MIR" Turbine<sup>®</sup>, Vacumed<sup>®</sup>, Ventura, CA, USA). The expired ventilation (V<sub>E</sub>), oxygen consumption per minute (VO<sub>2</sub>), and carbon dioxide produced per minute (V<sub>E</sub>) were sampled in breath-by-breath mode, where a measurement was generated every 5 breaths using specific software (Vista Turbo Fit<sup>®</sup> 5.1, Ventura, CA, USA). Heart rate was monitored continuously via telemetry.

Blood samples of 25 µl were collected by puncture of a hyperemic ear lobe for blood lactate measurements at the end of effort. The samples were immediately analyzed by an electroenzymatic method (YSI 1500 Sport L-Lactate Analyze®, Yellow Springs, USA). Total blood lactate was determined following hemolysis with Triton X-100 (YSI #1515 Agent Cell Lysing, USA) at 0.25%.

# **Controls and Calibration**

The gas analyzer, the lactate analyzer, and the CompuTrainer were calibrated before each test. The ergospirometer was calibrated by means of a certified mixture of gases containing 16% oxygen and 4.1% carbon dioxide with the rest nitrogen (Linde®, Rio de Janeiro, R.J., Brazil). The lactate analyzer was calibrated using a standard solution of 5 mmol·L<sup>-1</sup> lactate (YSI #2327 Lactate Standard YSI®, USA). Before each test and after every hour of use, a new calibration was performed. Before each experiment the linearity of the equipment was confirmed by means of a calibration curve with standards of 2.5, 5.0, 7.5, 15.0, and 30.0 mmol·L<sup>-1</sup>, prepared by the dilution of standards supplied by the manufacturer (YSI #2327: 5 mmol·L<sup>-1</sup>; YSI #2328: 15 mmol·L<sup>-1</sup>, YSI #1530: 30 mmol·L<sup>-1</sup> Lactate Standard YSI®, USA). The association between measured and expected values in the calibration curve was:  $r^2 = 0.9997$ , y = 0.9607x + 0.2071 and SEE = 0.1982 mmol·L<sup>-1</sup>, and the association between the first and second measurements was:  $r^2 = 1.0000$ , y = 1.0054x + 0.0161 and SEE = 0.0421 mmol·L<sup>-1</sup>.

The CompuTrainer was calibrated before each test. The rear tire used in the electromagnetic ergometer training device was set to 100 psi. A rolling resistance calibration program was used to measure the rolling resistance. Warm-up of the tire and the load generator was carried out to obtain temperature equilibrium, since the rolling resistance decreases as system temperature increases (10). Warm-up was conducted for 10 min by increasing the load until a power of 150 watts was attained. After the warm-up, the calibration program was activated and the display showed the letter "U" (uncalibrated) and the number 2.00 (2 lbs), demonstrating that the equipment was uncalibrated. Pedaling was restarted and when the speed reached 12 mi·hr<sup>-1</sup>, the word "UP" appeared on the display as a signal to pedal faster. When the speed reached 25 mi·hr<sup>-1</sup> the word "UP" disappeared from the screen, pedaling was stopped, and the rolling resistance was measured. The calibration was repeated two or three times until the difference between the values was minimal (0.05 to 0.10 lbs). The values were saved and the calibration was finished.

### **Statistical Analyses**

Statistical treatment was performed by means of the Statistical Package for the Social Sciences<sup>®</sup> (SPSS<sup>®</sup> Inc., Chicago, IL, USA), SigmaPlot<sup>®</sup> (Systat<sup>®</sup> Software Inc., Chicago, IL, USA) and Microsoft Excel<sup>®</sup> applications for Windows<sup>®</sup> (Microsoft<sup>®</sup>, Redmond, WA, USA). The descriptive statistics with average ± standard deviation (SD) were used.

Since the accuracy of gas analysis systems may vary according to the intensity of the exercise (14), the reliability was analyzed at different workloads. The rates observed in M1 (test) versus M2 (retest) were compared in each quartile of the workloads employed. Pearson's correlation coefficient was calculated for M1 versus M2 in each quartile and for each variable. The Fisher Z transformation was conducted to compare differences between correlations at different effort intensities for each variable.

Two-way repeated measures ANOVA with the Greenhouse-Geisser correction and the Tukey-HSD *post-hoc* tests were used to determine the significant differences between M1 (test) and M2 (retest), and between quartiles. The coefficient of determination ( $r^2$ ) and limits of agreement (Bland-Altman) for comparing the confidence interval of 95% were employed for the variables VO<sub>2</sub>, VCO<sub>2</sub>, and V<sub>E</sub> in M1 and M2. The degree of association between M1 and M2 was determined using the intraclass correlation coefficient (ICC). The error between test and retest was defined by the coefficient of variation (CV). All statistical tests were performed at a significance level of P≤0.05.

# RESULTS

Table 1 shows the values attained at the end of the two ergometric tests that characterized the maximum effort. The participants reached their predicted maximum effort both times. The values of  $VO_2$ ,  $VCO_2$ , and  $V_E$  were grouped into quartiles and maximum effort into test and retest (Table 2). As expected due to increased load during the test, significant differences were found (P<0.001) between quartiles for the three variables. However, there was no significant difference between M1 and M2 for any quartile. The correlations between test and retest were significantly greater at high intensities.

#### Table 1. Variables Attained at Maximum Effort.

	<b>M1</b> (Mean ± SD)	<b>M2</b> (Mean ± SD)
Blood Lactate (mmol <sup>·</sup> L <sup>-1</sup> )	8.65 ± 1.73	8.34 ± 1.96
%HR Max End	98.4 ± 4.24	99.1 ± 4.28
RER	1.08 ± 0.05	$1.09 \pm 0.06$

M1 – Test, M2 – Retest, HR End – Heart Rate attained at end of exercise, HR Max – Estimated Maximum Heart Rate (220 – age), %HR Max End – Percentage of HR max attained at the end of the test, RER – Respiratory Exchange Ratio.

Figure 1 (A – F) shows the relationship between M1 and M2 for each variable throughout the test. The panels on the right (B, D, and F) show the limits of agreement of Bland-Altman plots. The values for VO<sub>2</sub>, VCO<sub>2</sub>, and V<sub>E</sub> obtained in the retest were quite similar to those measured in the test with the slope and intercept respectively close to 1 and 0 for all three variables. The ICC was high and CV% was less than 6% for VO<sub>2</sub>, VCO<sub>2</sub>, and V<sub>E</sub> at all stages of the test (Table 3). Considering only the maximum effort, the CV% was 3.1, 3.8, and 5.3%, respectively.

	VO <sub>2</sub>		VCO <sub>2</sub>			V <sub>E</sub>			
	M1	M2	r	M1	M2	r	M1	M2	r
1st Quartile	2.32 ± 0.19	2.35 ± 0.27	0.68*	2.24 ± 0.23	2.24 ± 0.21	0.53*	51.36 ± 6.07	51.55 ± 4.30	0.66*
2nd Quartile	3.18 ± 0.34	3.25 ± 0.40	0.83*	$2.95 \pm 0.30$	2.94 ± 0.31	0.69*	66.22 ± 8.05	67.71 ± 7.77	0.75*
3rd Quartile	4.05 ± 0.28	4.12 ± 0.29	0.78*	3.80 ± 0.37	3.86 ± 0.36	0.78*#	89.57 ± 10.37	91.65 ± 10.07	0.86*#
4th Quartile	4.57 ± 0.33	4.65 ± 0.33	0.88*#	4.55 ± 0.44	4.71 ± 0.39	0.75*#	121.06 ± 31.23	124.57 ± 30.02	0.94*#
Max Effort	4.65 ± 0.46	4.74 ± 0.46	0.91*#	4.70 ± 0.37	4.81 ± 0.41	0.78*#	134.48 ± 24.17	133.79 ± 20.23	0.87*#
P (Quartile)	0.001	0.001		0.001	0.001		0.001	0.001	
P (M1 vs. M2)	0.1	100	0.232		0.295				

#### Table 2. Rate Observed during Incremental Exercise (Mean ± SD).

The quartile and maximum effort (max effort) values are presented as mean and standard deviation. M1 – test, M2 – retest. P (Quartile): comparison between quartiles; P (M1 vs. M2) value: P value of the comparison between M1 versus M2; r – Pearson's correlation coefficient. \*Significant correlation between M1 versus M2 for the different quartiles and for the max effort. \*Significant difference (P<0.05) for the correlation compared to the 1st Quartile by means of Fisher Z transformation.

#### DISCUSSION

This research arises from the importance of using an ergospirometric system that is both reliable and accurate in exercise physiology assessments and/or research. Accordingly, the objective was to evaluate the reliability of the stationary ergospirometer system Vista Mini-CPX<sup>®</sup> during a protocol for continuous scaled effort up to the cyclist maximum on an electromagnetically braked training ergometer. The results showed good reproducibility for the variables VO<sub>2</sub>, VCO<sub>2</sub>, and V<sub>E</sub>.

Cyclists perform best when the tests are performed in a posture similar to that used in training (15,31). One instrument that accommodates the cyclists' posture is the CompuTrainer (RacerMate, Inc., Seattle, WA, USA). An advantage of this equipment is that it can be coupled to the bike an athlete commonly uses in training and/or competition. Previous studies have verified the validity and reliability of CompuTrainer compared with other equipment (12,34). Peveler (32) recently demonstrated the reproducibility of CompuTrainer measurements in two identical indoor time trials, in which the test and retest showed similar results ( $34.74 \pm 8.54$  min and  $34.37 \pm 8.63$  min), thus confirming the reliability of this tool for laboratory purposes.

Reliability in measurements of gas exchange and ventilation rate is essential, which requires correct procedures for calibration, operation, and data analysis (4). According to Mendelsohn et al. (29), the reliability of the values generated by certain software during maximal exercise is limited. The American Thoracic Society (1) states that the reliability of the gas analyzers should be approximately 97%. In the present study, errors of 3.1 and 5.3% for test and retest were found. Jones and Kane (18) found errors of 3.8 and 8.0% for VO<sub>2</sub> and V<sub>E</sub>. However, the reliability of the outcome variables given

in a test is also influenced by physiological variability (16,21). According to Armstrong and Costill (3), when the correct procedures for calibration and operation are carried out, the biological and technical errors between test and retest represent on average 4.0% and 3.6%, respectively. Using 80 repeated trials with Douglas bags, Katch et al. (19) reported the total within-subject variation to be 5.6%, corresponding to more than 90% of this variability, while technical error accounted for less than 10%. Macfarlane and Wu (27) show the within subject error to also be about 6%, so their technical error was about 30% of total error, much higher than 10% estimated of Katch et al. (19).

Reference	n	Conditions	System		ICC	<b>CV</b> (%)	Average Difference (± 95%)
Present study	10	Cycloergometer: INC	Vacumed mini vista	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>	0.98 0.96 0.98	3.9 5.4 5.7	-0.05 (± 0.38) -0.07 (± 0.51) -1.51 (± 13.15)
Lothian et al. 1993	1	Treadmill: INC	Quinton	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>		1.1 - 3.9	
Meyer et al. 2001	23	Cycloergometer: INC	MetaLyz er 3B	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>	0.97 0.96 0.95		-0.01 (± 0.40) -0.01 (± 0.40) 0.01 (± 13.00)
Carter and Jeukendrup, 2002	10	Cycloergometer: SUBMÁX 100 W 150 W	Douglas Bag	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>		3.3 - 5.1 3.9 - 5.0 5.1 - 5.7	
Carter and Jeukendrup, 2002	10	Cycloergometer: SUBMÁX 100 W 150 W	Oxycon Alpha	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>		4.5 - 6.3 4.8 - 5.3 6.1 - 7.3	
Carter and Jeukendrup, 2002	10	Cycloergometer: SUBMÁX 100 W 150 W	Pulmolab EX670	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>		26.8 - 33.6 33.1 - 45.8 7.6 - 12.5	
Carter and Jeukendrup, 2002	10	Cycloergometer: SUBMÁX 100 W 150 W	Oxycon Pro	$\begin{matrix} VO_2 \\ VCO_2 \\ V_E \end{matrix}$		4.7-6.5 5.3-7.1 6.6-7.4	
Crouter et al. 2006	10	Cycloergometer: INC	TrueOne 2400	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>	0.99 0.99 0.96	4.7 5.7 7.3	-0.04 (± 0.23) -0.03 (± 0.28) -1.34 (± 10.56)
Crouter et al. 2006	10	Cycloergometer: INC	Douglas Bag	VO <sub>2</sub> VCO <sub>2</sub> V <sub>E</sub>	0.99 0.99 0.97	5.3 6.0 8.5	-0.05 (± 0.28) -0.05 (± 0.33) -1.36 (± 11.22)

#### Table 3. Previous Studies on Reliability of Stationary Equipment.

**n** - Number of Subjects, **ICC** - Intraclass Correlation Coefficient, **CV** - Coefficient of Variation, **INC** – Increment Test, SUBMAX - Test SUBMAXimal Fixed Load.



Figure 1. Comparison of values for VO<sub>2</sub> (A;B), VCO<sub>2</sub> (C;D), and V<sub>E</sub> (E;F) obtained in M1 and M2. The panels at left show the regression line (solid line) and line of identity (dashed line), regression equation, coefficient of determination ( $r^2$ ) and standard error of estimate (SEE). The panels at right show the limits of agreement of Bland-Altman (BA). The solid lines refer to the average of the differences and the dashed lines refer to the variation, ± 1.96 SD.

Several studies on the reliability of gas analyzers with stationary systems have been reported (5,8,16,24,25,30) (refer to Table 3). There is considerable variation among different ergometric devices and protocols (35), especially when the exercise intensity is one of the variables. We compared the total error (biological error and technical error) during a test with increasing load and subdivided the results into quartiles. This approach allowed us to determine whether the equipment showed better reliability at lower or higher loads and whether it remained stable throughout the test. The reliability was significant during the entire test, but was greater with increase effort (see Table 2).

### CONCLUSIONS

The findings indicate that there was no significant difference (P>0.05) between test and retest measurements on the device at different intensities. Measurements of test and retest of each respiratory variable were significantly correlated, with  $r^2 = 0.95$ , 0.90, and 0.95 and intraclass correlation coefficient 0.98, 0.96, and 0.98 VO<sub>2</sub>, VCO<sub>2</sub>, and V<sub>E</sub>, respectively. The Vista Mini-CPX®, Vacumed® system can be considered a reliable tool to measure and assess the cardiorespiratory performance of subjects in a laboratory or research setting.

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