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A Rapidly-incremented Tethered-swimming Maximal Protocol for Cardiorespiratory Assessment of Swimmers --Manuscript Draft--

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TITLE:**A Rapidly incremented Tethered-Swimming Maximal Protocol for Cardiorespiratory Assessment of Swimmers****AUTHORS AND AFFILIATIONS:**

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KEYWORDS:

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SUMMARY:

As opposed to measurement during free swimming, which presents inherent challenges and limitations, determination of important parameters of cardiorespiratory function for swimmers can be made using a more feasible and easier to administer tethered-swimming rapidly incremented protocol with gas exchange and ventilatory data collection.

ABSTRACT:

Incremental exercise testing is the standard means of assessing cardiorespiratory capacity of endurance athletes. While the maximal rate of oxygen consumption is typically used as the criterion measurement in this regard, two metabolic breakpoints that reflect changes in the dynamics of lactate production/consumption as the work rate is increased are perhaps more relevant for endurance athletes from a functional standpoint. Exercise economy, which represents the rate of oxygen consumption relative to performance of submaximal work, is also an important parameter to measure for endurance-athlete assessment. Ramp incremental tests comprising a gradual but rapid increase in work rate until the limit of exercise tolerance is reached are useful for determining these parameters. This type of test is typically performed on a cycle ergometer or treadmill because there is a need for precision with respect to work-rate incrementation. However, athletes should be tested while performing the mode of exercise required for their sport. Consequently, swimmers are typically assessed during free-swimming incremental tests where such precision is difficult to achieve. We have recently suggested that stationary swimming against a load that is progressively increased (incremental tethered swimming) can serve as a “swim ergometer” by allowing sufficient precision to accommodate a gradual but rapid loading pattern that reveals the aforementioned metabolic breakpoints and exercise economy. However, the degree to which the peak rate of oxygen consumption achieved during such a protocol approximates the maximal rate that is measured during free swimming remains to be determined. In the present article, we explain how this rapidly incremented tethered-swimming protocol can be employed to assess the cardiorespiratory capacity of a swimmer. Specifically, we explain how assessment of a short-distance competitive swimmer using this protocol revealed that his rate of oxygen uptake was 30.3 and 34.8 mL·min⁻¹·kg⁻¹BM at his gas-exchange threshold and respiratory compensation point, respectively.

INTRODUCTION:

An exercise test that involves an incremental increase in work rate (WR) from low to maximal (i.e., incremental exercise test; INC) provides the gold standard method of cardiorespiratory assessment for endurance athletes. In addition to the highest WR that the athlete can achieve (WR_{peak}), INC also allows for determination of the highest rate at which the individual can consume oxygen (O₂) for that form of exercise ($\dot{V}O_{2peak}$) if gas exchange and ventilatory data are collected during the test¹. The $\dot{V}O_{2peak}$ represents the criterion measure of cardiorespiratory fitness. Moreover, analysis of gas exchange and ventilatory data collected as WR is increased provides a non-invasive way to identify the point at which blood-lactate concentration (blood [lactate]) increases above the baseline value (lactate threshold) and the point at which it begins to accumulate at an accelerated rate (lactate turnpoint)². These metabolic breakpoints are estimated by determining the gas-exchange threshold (GET) and respiratory-compensation

point (RCP), respectively³. Importantly, the GET provides a robust estimate of the point at which blood [lactate] initially increases whereas the “hyperventilation” that characterizes RCP is a more complex phenomenon that can be initiated by afferent input other than chemoreception per se. Consequently, conclusions based on identification of RCP should be made with caution.

When exercise is maintained at a constant rate of work (CWR), there are markedly different physiological response profiles based on the “exercise-intensity domain” within which the WR falls^{4,5}. Specifically, achievement of a $\dot{V}O_2$ and blood [lactate] “steady state” is rapid in the moderate domain, delayed in the heavy domain and unattainable in the severe domain^{4,5}. It is well established that the rate at which O_2 can be consumed at GET during INC ($\dot{V}O_{2GET}$) serves as the metabolic rate that separates the moderate from heavy domain during CWR^{3,6}. Although controversial, a number of recent observations indicate similar equivalence between the rate at which O_2 can be consumed at RCP ($\dot{V}O_{2RCP}$) and heavy/severe separation⁷⁻¹⁰. Identification of $\dot{V}O_{2GET}$ and $\dot{V}O_{2RCP}$ from data collected during INC might, therefore, be useful for prescribing domain-specific training regimens for endurance athletes via metabolic rate with the caveat that aligning a metabolic rate with a specific work rate is more complex than simply doing so according to the $\dot{V}O_2$ -work rate relationship derived from the incremental test^{8,11}.

When the concept of testing to determine $\dot{V}O_{2max}$ was initially explored, researchers had subjects perform bouts of track running to the limit of exercise tolerance (T_{lim}) at increasing speeds on separate days¹. Research followed which confirmed that $\dot{V}O_{2max}$ can also be determined from similar bouts performed to T_{lim} on the same day with rest periods interspersed¹². Eventually, it was shown that a continuous protocol with WR increased in an incremental manner at specific time intervals (e.g., every 3 min) revealed the same $\dot{V}O_{2peak}$ as the discontinuous tests¹³. Consequently, these “graded exercise tests” became the standard for determining this criterion measure of cardiorespiratory fitness. However, in 1981, Whipp and colleagues published research that indicated that for the purpose of $\dot{V}O_{2max}$ measurement, INC could also be performed entirely in the non-steady state; that is, with WR increasing continuously as a “smooth function of time” (RAMP-INC)¹⁴. Unlike INC with extended stages and relatively large WR increases per stage, the gradual increase during RAMP-INC ensures that the “isocapnic buffering region” that separates GET and RCP will be clearly defined¹⁵. Furthermore, much like INC with stages, RAMP-INC can be used to assess “exercise economy” (i.e., the $\dot{V}O_2$ required per given WR); however, unlike INC with stages, in this case, it is the *inverse* of “delta efficiency” (i.e., the slope of the $\dot{V}O_2$ -WR relationship) that is used for this purpose¹¹ with consideration given to the fact that due to the complexities of the $\dot{V}O_2$ response to work rates across the intensity spectrum, this parameter will not be an immutable feature of INC per se (e.g., RAMP-INC initiated from different baseline work rates or characterized by different ramp slopes) or CWR exercise¹⁶.

For general fitness testing, INC is usually performed on a leg ergometer or treadmill because these modalities are more available and leg cycling and walking/running are familiar to the average person. Moreover, administration of RAMP-INC requires the ability to increase WR continuously in small increments (e.g., 1 W every 2 s); hence, an ergometer (typically leg

cycling) is best suited for this type of testing. However, athlete assessment is more complex because athletes must be tested while performing the specific mode of exercise required for their sport. For cyclists and individuals who participate in sports that involve running, this is not problematic because of the accessibility and applicability of the aforementioned testing machines. Conversely, ecologically-valid testing with gas exchange and ventilatory data collection and the gradual WR incrementation required for RAMP-INC is more challenging when assessing aquatic athletes.

Prior to the advent of automated collection systems, gas-exchange assessment of swimmers was often performed using Douglas-bag collection following a maximal swim¹⁷. Once automated systems were developed, “real-time” collection took place, but not under “real-swimming” conditions (e.g., while swimmers swam in a flume which controlled WR)¹⁷. Unfortunately, the former method has inherent limitations due to the assumptions of “backward extrapolation” while the latter raises concerns regarding the degree to which flume swimming changes technique¹⁷. The current state of the art involves the use of portable breath-by-breath collection systems which move with the swimmer alongside the pool during free swimming¹⁷. While this type of measurement improves ecological validity, gradual WR incrementation is challenging. Indeed, INC during free swimming typically involves intervals of set distance (e.g., 200 m) at progressively-increasing velocities^{14,15}. This means that a test consists of lengthy stages with large unequal WR increments. It is, therefore, not surprising that only a single metabolic breakpoint (typically called the “anaerobic threshold”) is reported by researchers who employ this test^{18,19}. Instead, we have recently shown that both $\dot{V}O_{2GET}$ and $\dot{V}O_{2RCP}$ can be determined from data collected while swimmers performed stationary swimming in a pool against a load that was increased gradually and rapidly (i.e., incremental tethered swimming)²⁰. While the unique breathing pattern that is present during swimming might render the aforementioned breakpoints harder to identify compared to typical modes of assessment (personal observation), we believe that this method of testing might be suitable as a “swim ergometer” that can be used for cardiorespiratory assessment of swimmers in a manner similar to how a stationary cycle is used for cyclists. Indeed, we have shown that $\dot{V}O_{2GET}$, $\dot{V}O_{2RCP}$ and exercise economy (as indicated by the $\dot{V}O_2$ -load slope) can all be determined from the rapidly incremented tethered-swimming protocol that is described below²⁰.

PROTOCOL:

Participants in the study from which the representative-subject data presented below were extracted²⁰ ($n = 11$) were required to give their written informed consent prior to initiation of testing after the experimental procedures, associated risks and potential benefits of participation had been explained. The first visit comprised a familiarization session during which the swimmers were introduced to the concept of tethered swimming and the measurement techniques that would be in effect during the actual testing. An all-out tethered-swimming test was performed during the second visit and the rapidly incremented tethered-swimming protocol was performed on the third visit. Both tests were done in a semi-Olympic pool (25 m) with water temperature at 28 ° C.

1. Preparation of swimmer

- 177
- 178 1.1. Instruct the swimmer to avoid strenuous exercise for 24 h preceding each testing session.
- 179
- 180 1.2. Instruct the swimmer to arrive at the pool in a rested and fully hydrated state ≥ 3 h
- 181 postprandial.
- 182
- 183 1.3. Instruct the swimmer to refrain from ingesting stimulant beverages and alcohol for 24 h prior
- 184 to each test.
- 185

186 **2. All-out tethered-swimming test**

187

188 2.1. Prepare the 500 kg load cell that will be used to measure the highest force that the swimmer

189 can exert during two trials comprising 30 s of all-out swimming²¹.

190

191 2.1.1. Open the N2000PRO Software (Power Din Pro - CEFISE) program on the computer.

192

193 2.1.2. Open the **Help Menu** to verify the communication link between the computer and the load-

194 cell analyzer.

195

196 2.1.2.1. Observe a green signal that indicates that the connection to the RS232 interface is well

197 established.

198

199 2.1.2.2. Set the countdown to start the test depending on circumstances.

200

201 2.1.2.3. Set the sampling duration. Set the rest interval. Set the frames per second at 100 Hz.

202

203 2.1.2.4. Set the unit of force measurement at N or kg depending on personal preference. Set

204 the acquisition time in milliseconds.

205

206 2.1.3. Calibrate the load cell²² with 0 and 10 kg loads with the swimmer outside pool.

207

208 2.1.4. Attach a load cell to the start block via the L-shaped flattened iron bar that is designed by

209 CEFISE specifically for tethered-swimming measurements.

210

211 2.1.5. Attach one end of the inelastic rope to the load cell and the other end to the swimmer by

212 means of the custom-designed belt (CEFISE), which has ropes attached to both hips such that leg

213 kicking will not interfere with the force measurement.

214

215 2.2. Prepare the swimmer for performance of the two-trial test.

216

217 2.2.1. Provide instructions to the swimmer regarding correct performance of all-out front-crawl

218 swimming (e.g., prevent the head and trunk from rising while swimming as rapidly as possible,

219 concentrate on kicking at a maximal rate in addition to maximal stroking, etc.).

220

- 2.2.2. Instruct the swimmer to perform stretching and arm/leg swings at poolside in preparation.
- 2.2.3. Instruct the swimmer to enter the pool and perform a standard warm-up protocol comprised of front-crawl swimming for 800 m at a light intensity with care taken to avoid engendering any lingering effects that could influence the results of the test .
- 2.2.4. Allow the swimmer to exit the pool and rest at poolside for 10 min.
- 2.2.5. Secure the belt around swimmer's waist. Attach the free end of the inelastic rope to the belt.
- 2.2.6. Determine the load required to maintain the swimmer's body horizontally with a minimum amount of tension on the measurement system ($load_{base}$).
- 2.2.7. Signal the swimmer to begin Trial #1 of the test.
- 2.3. Monitor the swimmer during performance of the test.
- 2.3.1. Provide verbal encouragement to swimmer throughout 30 s test.
- 2.3.2. Signal the swimmer to end the test. Detach the swimmer from the inelastic rope.
- 2.3.3. Instruct the swimmer to perform a standard cool-down protocol comprised of front-crawl swimming at a light intensity.
- 2.3.4. Allow the swimmer to rest for 30 min at poolside.
- 2.3.5. Reattach the swimmer to the inelastic rope.
- 2.3.6. Signal the swimmer to start Trial #2 of the test which is identical to Trial #1 (30 s of all-out swimming).
- 2.3.7. Signal the swimmer to end the test.
- 2.3.8. Instruct the swimmer to perform a standard cool-down protocol comprised of front-crawl swimming at a light intensity.
- 2.3.9. Allow the swimmer to exit pool.
- 2.4. Analyze the data collected during the two-trial test.
- 2.4.1. Apply the smoothing process to the data using the N2000PRO software package²³.
- 2.4.2. Calculate the peaks of the wave-frequency signal from the force-time sinusoidal curve (range, sine 80 °–100 °) above $load_{base}$ for Trials #1 and 2.

265
266 2.4.3. Define the averaged peaks of the force-time wave-frequency signal in the first 5 s and entire
267 30 s, respectively, as the peak force (F_{peak}) and average force (F_{avg}) for each trial.

268
269 2.4.4. Use the higher values for F_{peak} and F_{avg} for further calculations.

270 271 **3. Incremental tethered-swimming test**

272
273 3.1. Calculate the loads that will be used to resist the swimmer's forward displacement during the
274 incremental test.

275
276 3.1.1. Calculate the starting load as 30% of the F_{avg} above $\text{load}_{\text{base}}$.

277
278 3.1.2. Calculate the increments to be applied per 60-s stage as 5% of F_{avg} above $\text{load}_{\text{base}}$.

279 280 **3.2. Prepare the automated portable metabolic unit for data collection.**

281
282 3.2.1. Open the unit's software.

283
284 3.2.2. Verify the communication link between the computer and the automated portable metabolic
285 unit.

286
287 3.2.3. Power on unit and allow to warm up for 45 min. Ensure that batteries are fully charged.

288
289 3.2.4. Perform calibration of unit for environmental air²⁴.

290
291 3.2.5. Perform calibration of unit for reference O_2 (16%), CO_2 (5%) and N (balance) concentrations²⁴.

292
293 3.2.6. Perform mask time-delay calibration²⁴.

294
295 3.2.7. Perform calibration of turbine with 3 L syringe²⁴.

296
297 3.2.8. Enter the subject data, ambient temperature and humidity.

298 299 **3.3. Prepare the swimmer for performance of the incremental test.**

300
301 3.3.1. Install a facemask and a snorkel on the swimmer.

302
303 3.3.2. Instruct the swimmer to rest at poolside for 10 min to collect "baseline" gas exchange and
304 ventilatory data.

305
306 3.3.3. Instruct the swimmer to enter the pool and perform a standard warm-up protocol comprised
307 of front-crawl swimming at a light intensity.

308

3.3.4. Secure a belt around the swimmer's waist. Attach an inelastic rope to the belt with the other end of rope attached to the loading system.

3.3.5. Instruct the swimmer that once the test begins to use the two markers on the bottom of the pool for reference points, which allow them to maintain a relatively-fixed position (e.g., ± 1 m from the desired position).

3.3.6. Signal the swimmer to begin the test.

3.4. Monitor the swimmer during performance of the incremental test.

NOTE: A research assistant who is experienced in monitoring this type of testing should hold the gas-analysis unit at poolside being cognizant to do so without impeding swimmer displacement and/or elevating the swimmer's head.

3.4.1. Increase load while timing the 60 s stages.

3.4.2. Terminate the test and record the time to limit of exercise tolerance when the swimmer is no longer able to maintain the requisite position despite strong verbal encouragement from the testers.

3.4.3. Use the time to limit of exercise tolerance to calculate stages completed.

3.4.4. Record loads for each stage and peak load.

3.4.5. Detach the swimmer from the inelastic rope.

3.4.6. Instruct the swimmer to perform a standard cool-down protocol comprised of front-crawl swimming at a low-to-moderate intensity.

3.4.7. Allow the swimmer to exit the pool.

3.5. Analyze the data collected during the incremental test.

3.5.1. Smooth breath-by-breath gas-exchange data that were collected before and during test using the unit's software program.

3.5.2. Export gas-exchange data in consecutive 9 s bin averages.

3.5.3. Perform three-point rolling average on consecutive 9 s bin averages for $\dot{V}O_2$.

3.5.4. Record highest three-point rolling-average value as the $\dot{V}O_{2peak}$.

3.5.5. Using final three-point rolling-average value for each completed stage, calculate $\dot{V}O_2$ -load relationship via linear regression. Exclude data from end stages of test if a $\dot{V}O_2$ plateau appears to be present (visual inspection).

3.5.6. Using consecutive 9 s bin averages, determine $\dot{V}O_{2GET}$.

3.5.6.1. Determine the first disproportionate increase in the rate of CO_2 production ($\dot{V}CO_2$) compared to $\dot{V}O_2$.

3.5.6.2. Determine the increase in the ratio of the expired rate of ventilation (\dot{V}_E) to $\dot{V}O_2$ with no increase in the ratio of \dot{V}_E to $\dot{V}CO_2$.

3.5.6.3. Determine the increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension.

3.5.7. Using consecutive 9-s bin averages, determine $\dot{V}O_{2RCP}$.

3.5.7.1. Determine the first disproportionate increase in \dot{V}_E compared to $\dot{V}CO_2$.

3.5.7.2. Determine the decrease in end-tidal CO_2 .

3.5.8. Express $\dot{V}O_{2peak}$, $\dot{V}O_{2GET}$, $\dot{V}O_{2RCP}$ and $\dot{V}O_2$ -load slope in both absolute ($L \cdot min^{-1}$) and relative (to body mass; $mL \cdot min^{-1} \cdot kg^{-1}$) terms.

3.5.9. Express $\dot{V}O_{2GET}$ and $\dot{V}O_{2RCP}$ in relative terms as a percentage of $\dot{V}O_{2peak}$.

REPRESENTATIVE RESULTS:

The data presented in **Table 1** and depicted in **Figures 1–4** represents the response profiles observed for a male swimmer (age, 24 years). At the time of data collection, the swimmer had been training for competitive swimming for 7 years. His specialty was short-distance (i.e., 50 m and 100 m) freestyle events.

The initial load on INC was set at a load that exceeded that which was required for this swimmer to maintain body alignment prior to initiation of the all-out swim (F_{base}) by 30% of the difference between the average force measured during the all-out swim and F_{base} (ΔF). For this swimmer, that load was 4.17 kg. The load was then increased by 0.7 kg for every 60 s stage (**Figure 1**). The limit of exercise tolerance for this swimmer occurred at 576 s (stage 10).

When breath-by-breath $\dot{V}O_2$ data collected during the baseline and exercise portions of INC were averaged into consecutive 9 s bins, the highest three-point rolling average was $3.44 L \cdot min^{-1}$ ($40.6 mL \cdot min^{-1} \cdot kg^{-1} BM$) (**Figure 2**) and the $\dot{V}O_2$ -load slope was $261 mL \cdot min^{-1} \cdot kg^{-1}$ ($3.1 mL \cdot min^{-1} \cdot kg^{-1} BM \cdot kg^{-1}$) (**Figure 3**). Identification of GET and RCP were made by consensus from a panel of independent reviewers experienced at making these determinations from a cluster of measurements. Being that GET represents the point during INC at which ventilation changes due to metabolic acidosis and the increased $\dot{V}CO_2$ that occurs consequent to its buffering (i.e., passage of the “lactate threshold”), it can

be identified by an increase in the ratio of both $\dot{V}CO_2$ and \dot{V}_E to $\dot{V}O_2$ that causes end-tidal O_2 to increase. However, for data collected during INC with sufficient sensitivity, this change in gas-exchange and ventilatory response profile will not be accompanied by a decline in end-tidal CO_2 because the increase in $\dot{V}CO_2$ and \dot{V}_E will be in proportion. Consequently, the partial pressure of CO_2 in arterial blood (P_aCO_2) will remain constant (i.e., isocapnic buffering; iso = same, capnic = CO_2) (**Figure 4**). Indeed, during rapid-incremental INC, the decline in P_aCO_2 and end-tidal CO_2 that characterizes “respiratory compensation” in response to metabolic acidosis will not occur for ≥ 2 min during which work (and metabolic) rate continue to increase (**Figure 4**)¹². For this swimmer, the metabolic rates characterizing these distinct changes in gas-exchange and ventilatory response driven by the increased contribution of the “anaerobic pathway” to energy demand occurred at 75% and 86% of the $\dot{V}O_{2peak}$, respectively (**Table 1**).

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic representation of the rapidly incremented tethered-swimming test that can be used to determine important parameters of cardiorespiratory fitness for swimmers. The loading profile and time to limit of exercise tolerance that are depicted are for a representative subject, a 24-year-old swimmer who competes in short-distance events.

Figure 2: Pulmonary O_2 uptake response during the rapidly incremented tethered-swimming test performed by the representative subject. The vertical dashed line represents the point at which load incrementation began. The red circles represent the highest three-point rolling-average rate of O_2 uptake that was measured during the test. We defined that value as this swimmer’s $\dot{V}O_{2peak}$ for this form of incremental exercise.

Figure 3: The $\dot{V}O_2$ -load coordinates for the representative subject for each completed stage of the rapidly incremented tethered-swimming test. Linear regression analysis was used to derive the line of best fit that is depicted. The slope of this line is used as a measure of exercise economy.

Figure 4: Gas exchange and ventilatory responses for the representative subject during the rapidly incremented tethered-swimming test. From left to right, vertical dashed lines are aligned with the gas exchange threshold and respiratory compensation point, respectively. Horizontal dashed lines are positioned at the nadir (top three graphs) or apex (bottom graph) of data points. See text for details on how these metabolic breakpoints were determined by visual inspection. With slight modification, this figure has been reprinted with permission from the original publishers²⁰.

Table 1: Cardiorespiratory parameters for the representative subject as measured during the rapidly incremented tethered-swimming test.

DISCUSSION:

An exercise challenge that involves enduring an incremental increase in WR until T_{lim} is reached is a standard testing protocol for assessment of endurance athletes. When such a test is performed with

gradual, but rapid incrementation, it is particularly useful because in addition to the $\dot{V}O_{2\max}$, gas exchange and ventilatory data collected during the test can be used to distinguish the region bounded by GET and RCP where acidosis is present, but arterial partial pressure of CO_2 (P_aCO_2) is maintained^{14,15}. The metabolic rates that serve as lower^{3,6} and upper⁷⁻¹⁰ boundaries of this region approximate those that partition the heavy-intensity domain during CWR.

Generally speaking, the primary parameter of interest derived from the assessment of endurance athletes with INC is the $\dot{V}O_{2\max}$, which is used to monitor the athlete's level of cardiorespiratory fitness. Moreover, $\dot{V}O_{2\max}$ is often used as a way to assign exercise WR when prescribing training programs (i.e., WR specified as a percentage of $\dot{V}O_{2\max}$). However, a growing body of research confirms that the pulmonary gas exchange (and, by extension, muscle metabolic) response to a linear increase in WR is not linear and, importantly, the characteristics of this non-linearity vary for different individuals (and for the same individual at different levels of conditioning)¹¹. Normalizing exercise intensity according to $\dot{V}O_{2\max}$ is, therefore, flawed because it does not guarantee a similar level of "metabolic strain" for different individuals^{11,26,27}. Conversely, normalizing intensity in relation to the intensity domains that reflect the nonlinearity across the intensity spectrum ensures that a similar metabolic challenge will be encountered. As opposed to $\dot{V}O_{2\max}$, it is, therefore, the metabolic rates that bound the exercise-intensity domains that are important to consider when the objective is to prescribe endurance training in a consistent fashion.

During CWR, metabolic rates situated below $\dot{V}O_{2GET}$ comprise the moderate-intensity domain where a $\dot{V}O_2$ steady state can be attained rapidly, muscle metabolic perturbation is minimal and exercise is sustainable for an extended period (e.g., ~4 h)^{4,5}. Within this domain, depletion of muscle [glycogen] and impairment in neuromuscular excitability/transmission have recently been implicated as reasons for reaching T_{lim} ⁵. For metabolic rates above $\dot{V}O_{2GET}$ but below what has been termed the "critical metabolic rate," a steady state $\dot{V}O_2$ is also achievable; however, in this case, attainment is delayed by the presence of a $\dot{V}O_2$ slow component that increases the $\dot{V}O_2$ cost of work above that which would be predicted by linear extrapolation of the $\dot{V}O_2$ cost of exercise in the moderate-intensity domain²⁸. During exercise within this domain, muscle metabolic perturbation (e.g., decreased [phosphocreatine], [ATP], [glycogen] and pH; increased [lactate]) is greater and T_{lim} is markedly reduced (e.g., ~45 minutes)⁵. A $\dot{V}O_2$ slow component is also present during CWR requiring metabolic rates above the critical metabolic rate (i.e., within the severe-intensity domain); however, in this case, a steady state cannot be achieved as $\dot{V}O_2$ rises inexorably, $\dot{V}O_{2peak}$ intervenes (if exercise is sustained for a long enough period of time), a critical level of substrate depletion and/or metabolite accumulation is reached and T_{lim} is imminent in a relatively short period of time (e.g., 2–14 min depending upon work rate)⁵.

With respect to endurance training for athletes, it is well accepted in both theory and practice that time should be devoted to exercise in each of the intensity domains so that positive adaptations exclusive to work performed in each can be gleaned²⁸. For example, a typical week for an endurance athlete might include easy training in the moderate domain, steady training in the heavy domain and tempo and interval training in the severe domain²⁹. With respect to prescribing exercise in such a domain-specific manner, the recognition that $\dot{V}O_{2GET}$ separates the moderate from heavy domain is well accepted^{3,6}; hence, moderate-intensity exercise can be prescribed in a normalized manner as a

percentage of $\dot{V}O_{2GET}$ as measured on a rapidly incremented RAMP-INC. Alternatively, controversy exists regarding the critical metabolic rate that establishes the heavy/severe border. Traditionally, the determination of the highest speed or power output that does not cause a rise of blood [lactate] of $> 1 \text{ mmol}\cdot\text{L}^{-1}$ between 10 and 30 min during a series of CWR bouts (i.e., the “maximal lactate steady state;” MLSS) has been used for this purpose^{30,31}. However, when actual measurements of T_{lim} are made by expending the finite capacity for work in the severe domain (W') during a series of CWR bouts or a single all-out bout, it has recently been suggested that the “critical power” (CP) so revealed (i.e., the asymptote of the power- T_{lim} hyperbola for the former testing protocol or the end-test power for the latter) can be greater than the power output indicated by the MLSS assessment³²⁻³⁵. At present, it is safe to conclude that while both CP and MLSS testing provide reasonable estimates of the heavy-/severe-intensity boundary, each of these estimates can be influenced by a number of factors such that congruence between the two is not always present.

In 2017, Keir et al. had subjects perform a multi-bout CP-testing protocol and found that the metabolic rate at CP was statistically similar to the RAMP-INC-derived measurement of $\dot{V}O_{2RCP}$ ⁷. The authors concluded that in addition to the metabolic rate at CP, $\dot{V}O_{2RCP}$ might provide an alternative way to determine the critical metabolic rate that separates the heavy from severe domain. However, it is important to recognize that if agreement is present, it is only when the parameters are expressed as metabolic rates because RCP cannot be linked to a specific work rate³⁶. Furthermore, given that respiratory compensation can be driven by both exercise WR (i.e., intensity) and the time for which a supra-GET WR is sustained, determining GET and RCP as distinct breakpoints (as opposed to a single “anaerobic threshold” that effectively melds the two) requires INC with relatively rapid incrementation²⁰. The clear delineation between the two breakpoints for the representative-subject data we have presented (see **Figure 4**) verifies that the rapidly incremented tethered-swimming test we are advancing satisfies this criterion.

In addition to discrete values for $\dot{V}O_{2GET}$ and $\dot{V}O_{2RCP}$, we have shown that a rapidly incremented tethered-swimming test can be used with sufficient caveats (see above regarding how this value will be specific for a given RAMP-INC protocol and not necessarily indicative of the response that will be present during CWR exercise) to determine the athlete’s exercise economy as indicated by the $\dot{V}O_2$ -load slope during the test²⁰. This is an important attribute to assess because athletes who are more economical are advantaged during endurance performance. For example, cross-sectional studies indicate that trained athletes possess better exercise economy³⁷ while longitudinal studies confirm that exercise economy improves from training³⁸. Consequently, deriving this parameter for swimmers from a rapidly incremented tethered-swimming test could be useful both for predicting athletic potential prior to training and monitoring changes that occur as a result of it. However, in addition to recognition of the specificity of this parameter for RAMP-INC (see above), it is important to recognize that only data from the linear-rise portion of the $\dot{V}O_2$ response should be used for this purpose. Conversely, any data reflecting an initial lag in the $\dot{V}O_2$ response (the $\dot{V}O_2$ “mean response time”) and/or a $\dot{V}O_2$ plateau preceding T_{lim} should be excluded from the fit.

One important caveat to our contention that the tethered-swimming test we have described can serve as a “swim ergometer” for measuring cardiorespiratory parameters that are relevant for free swimming is that the degree to which the tethered methodology alters technique sufficiently to

dissociate the two requires further elucidation. For example, when reporting the highest $\dot{V}O_2$ measured during the test¹⁶, we are reticent to refer to it as the $\dot{V}O_{2max}$ because we did not have swimmers also perform a free-swimming INC in our study²⁰. Consequently, we cannot confirm that the $\dot{V}O_{2peak}$ during the tethered test is similar to that which is measured using a free-swimming protocol. Although a correlation between the two values has been established³⁹⁻⁴¹, previous research that compared the two has returned equivocal findings. For example, Bonen et al. reported $\dot{V}O_{2peak}$ values for free and tethered swimming that were similar and within the range of variation expected for repeat measurement of $\dot{V}O_{2max}$ ⁴⁰ whereas Magel and Faulkner found a lower value for tethered compared to free swimming⁴¹. The reason(s) for these disparate findings are unclear but might reflect the fact that local muscular fatigue and/or ventilatory distress intervened before $\dot{V}O_{2max}$ was reached for swimmers who were unaccustomed to tethered swimming in the latter study⁴². Regardless of this distinction, future research should be designed to compare $\dot{V}O_{2peak}$ values for tethered and free swimming during both INC and supramaximal severe-intensity CWR bouts to T_{lim} which are used for confirming that a $\dot{V}O_{2peak}$ measured during INC is indeed the maximum $\dot{V}O_2$ that can be achieved (i.e., “verification bouts”)⁴². Similarly, over the course of the entire test, it is possible that applying load in an incremental manner might result in different “adaptive strategies” by the swimmers in response to the increase of intensity compared to increasing velocity during free swimming. For example, the load might reach a level beyond which biomechanical changes are required that are unlike those that would allow for a more rapid stroke pattern as velocity is increased during free swimming. This could influence the $\dot{V}O_2$ -WR slope and/or estimation of $\dot{V}O_{2GET}$ and $\dot{V}O_{2RCP}$. More research comparing tethered with free swimming is required to provide insight in this regard.

Unlike the speed increases that are used to increment WR during free-swimming INC testing, we have shown that the load increases employed for tethered swimming allow for a gradual, but rapid increase in WR. Consequently, we advance this type of testing as a “swim ergometer” that can be used to determine $\dot{V}O_{2GET}$, $\dot{V}O_{2RCP}$ and exercise economy much like a cycle ergometer is used for performing a smooth ramp protocol¹⁴. We have also used this test to measure the peak $\dot{V}O_2$ response; however, how this value compares to the $\dot{V}O_{2max}$ that is typically assessed during free swimming remains to be resolved.

ACKNOWLEDGMENTS:

This work was supported by CIPER–Foundation for Science and Technology (FCT), Portugal (UID/DTP/00447/2019) and financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001", and to the São Paulo Research Foundation - FAPESP (PROCESS 2016/04544-3 and 2016/17735-1). authors would like to thank João Guilherme S. V. de Oliveira to the assistance in data sampling.

DISCLOSURES:

The authors have no disclosures to report.

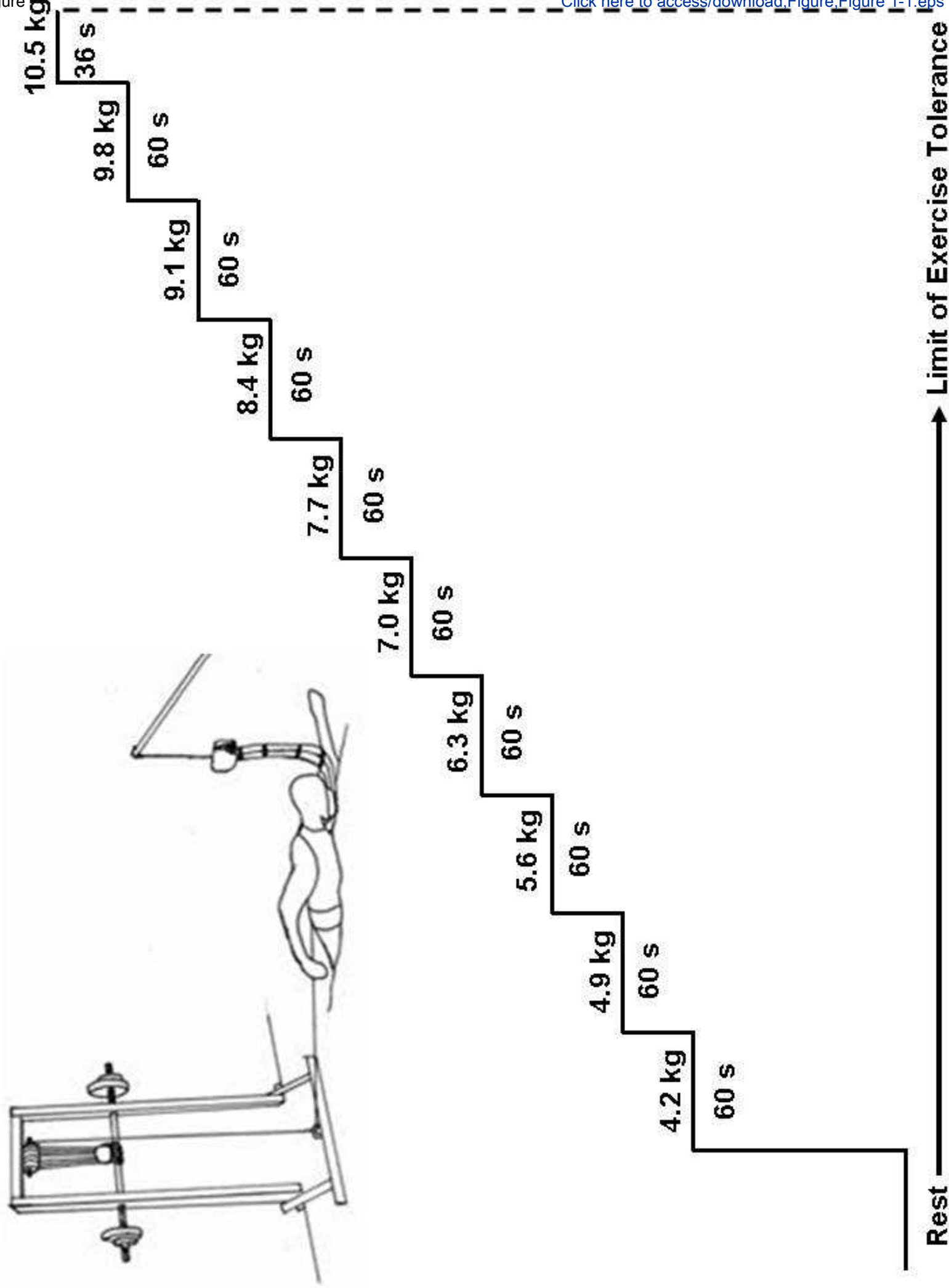
REFERENCES:

1. Hill, A.V. Lupton, H. Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Quarterly Journal of Medicine*. **16** (62), 135–171 (1923).

- 571 2. Davis, H.A., Bassett, J., Hughes, P. Gass, G.C. Anaerobic threshold and lactate turnpoint.
572 *European Journal of Applied Physiology Occupational Physiology*. **50** (3), 383–92 (1983).
- 573 3. Beaver, W.L, Wasserman, K. Whipp, B.J. A new method for detecting anaerobic
574 threshold by gas exchange. *Journal of Applied Physiology*. **60** (6), 2020–2027 (1986).
- 575 4. Whipp, B.J. Wasserman, K. Oxygen uptake kinetics for various intensities of constant-
576 load work. *Journal of Applied Physiology*. **33** (3) 351–356 (1972).
- 577 5. Black, M. I. et al. Muscle metabolic and neuromuscular determinants of fatigue during
578 cycling in different exercise intensity domains. *Journal of Applied Physiology*. **122** (3), 446–459
579 (2017).
- 580 6. Whipp, B.J. Dynamics of pulmonary gas exchange. *Circulation*. **76** (6 Pt 2), V118–128
581 (1987).
- 582 7. Keir, D.A. et al. Exercise Intensity Thresholds: Identifying the Boundaries of Sustainable
583 Performance. *Medicine and Science in Sports and Exercise*. **47** (9), 1932–1940 (2017).
- 584 8. Keir, D.A., Paterson, D.H., Kowalchuk, J.M. Murias, J.M. Using ramp-incremental $\dot{V}O_2$
585 responses for constant-intensity exercise selection. *Applied Physiology, Nutrition and*
586 *Metabolism*. **43** (9), 882–892 (2018).
- 587 9. Iannetta, D. et al. An equation to predict the maximal lactate steady state from ramp-
588 incremental exercise test data in cycling. *Journal of Science and Medicine in Sport*. **21** (12),
589 1274–1280 (2018).
- 590 10. Iannetta, D., Inglis, E.C., Fullerton, C., Passfield, L. Murias, J.M. Metabolic and
591 performance-related consequences of exercising at and slightly above MLSS. *Scandinavian*
592 *Journal of Medicine & Science in Sports*. **28** (12), 2381–2493 (2018).
- 593 11. DiMenna, F.J. Jones, A.M. “Linear” Versus “Nonlinear” $\dot{V}O_2$ Responses to Exercise:
594 Reshaping Traditional Beliefs. *Journal of Exercise Science & Fitness*. **7** (2), 67–84 (2009).
- 595 12. Mitchell, J.H., Sproule, B.J. Chapman, C.B. The physiological meaning of the maximal
596 oxygen intake test. *Journal of Clinical Investigation*. **37** (4), 538–547 (1958).
- 597 13. McArdle, W.D., Katch, F.I. Pechar, G.S. Comparison of continuous and discontinuous
598 treadmill and bicycle tests for max $\dot{V}O_2$. *Medicine and Science in Sports and Exercise*. **5** (3), 156–
599 160 (1973).
- 600 14. Whipp, B.J., Davis, J.A., Torres, F. Wasserman, K. A test to determine parameters of
601 aerobic function during exercise. *Journal of Applied Physiology: Respiratory Environmental and*
602 *Exercise Physiology*. **50** (1), 217–221 (1981).
- 603 15. Whipp, B.J., Davis, J.A. Wasserman, K. Ventilatory control of the ‘isocapnic buffering’
604 region in rapidly-incremental exercise. *Respiratory Physiology*. **76** (3), 357–367 (1989).
- 605 16. Boone J Bourgois J. The oxygen uptake response to incremental ramp exercise:
606 methodological and physiological issues. *Sports Medicine*. **42** (6), 511–526 (2012).
- 607 17. Sousa, A. et al. Critical evaluation of oxygen-uptake assessment in swimming.
608 *International Journal of Sports Physiology and Performance*. **9** (2), 190–202 (2014).
- 609 18. Fernandes, R.J., Sousa, M., Machado, L. Vilas-Boas, J.P. Step length and individual
610 anaerobic threshold assessment in swimming. *International Journal of Sports Medicine*. **32** (12),
611 940–946 (2011).
- 612 19. Ribeiro, J. et al. Metabolic and ventilatory thresholds assessment in front crawl
613 swimming. *The Journal of Sports Medicine and Physical Fitness*. **55** (7–8), 701–707 (2015).

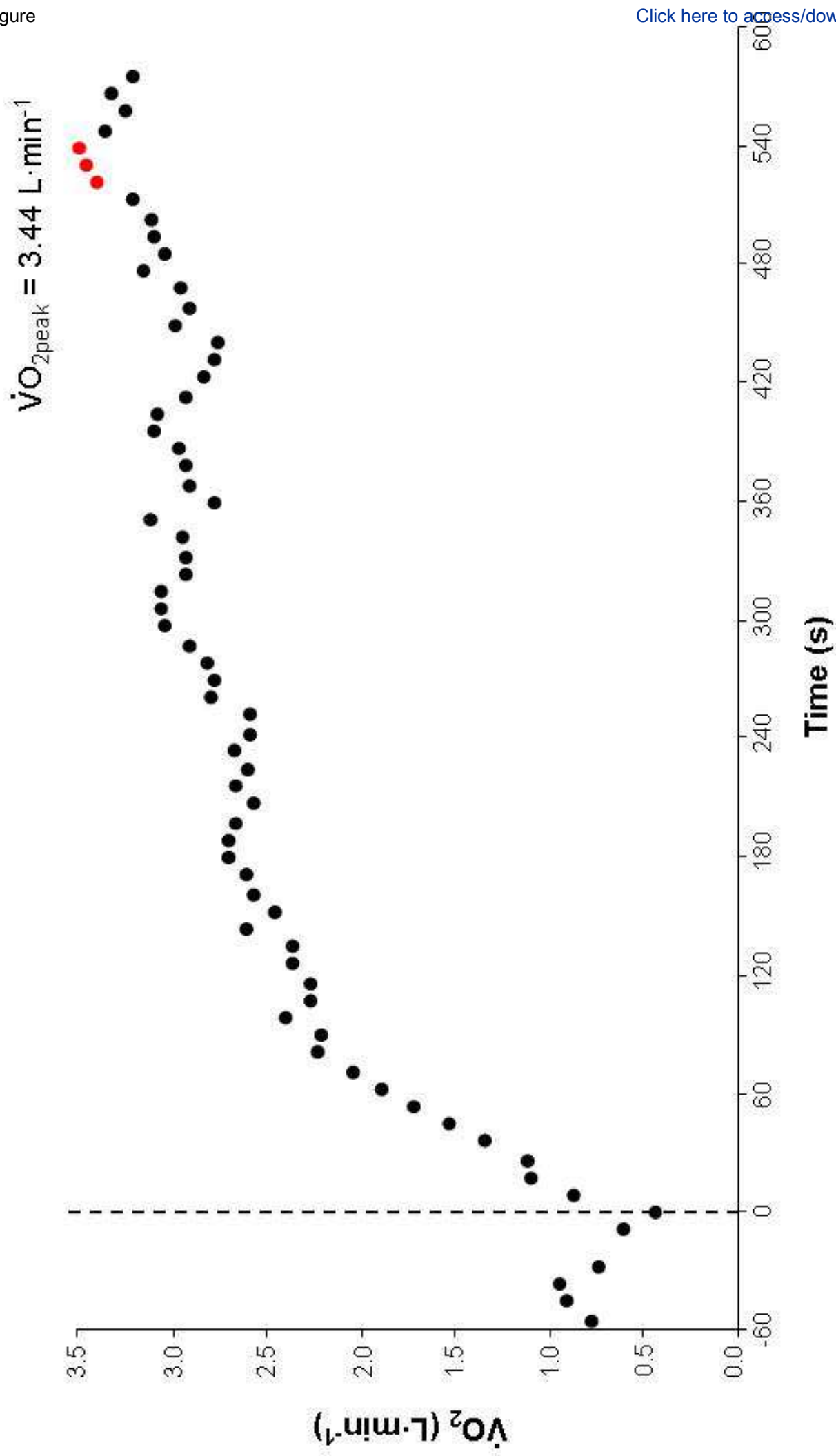
20. Pessoa Filho, D.M. et al. A rapidly incremented tethered-swimming test for defining domain-specific training zones. *Journal of Human Kinetics*. **57** (1), 117–128 (2017).
21. Dopsaj, M. et al. The relationship between 50m-freestyle results and characteristics of tethered forces in male sprint swimmers: A new approach to tethered swimming test. *Physical Education & Sport*. **1** (7), 15–22 (2000).
22. Cefise biotecnologia esportiva. <http://www.cefise.com.br/produtos/> (2019)
23. N2000 User Manual: http://www.cefise.com.br/anexos_manuais/90/Manual-N2000.pdf
24. Wheatley, C.M. et al. Conducting Maximal and Submaximal Endurance Exercise Testing to Measure Physiological and Biological Responses to Acute Exercise in Humans. *Journal of Visualized Experiments*. **17** (140) (2018).
25. Lansley, K.E, DiMenna, F.J., Bailey, S.J. Jones, A.M. A 'new' method to normalise exercise intensity. *International Journal of Sports Medicine*. **32** (7), 535–541 (2011).
26. Iannetta, D. et al. A Critical Evaluation of Current Methods for Exercise Prescription in Women and Men. *Medicine and Science in Sports and Exercise*. [Epub ahead of print] (2019).
27. Scharhag-Rosenberger, F., Meyer, T., Gässler, N., Faude, O. Kindermann, W. Exercise at given percentages of VO₂max: heterogeneous metabolic responses between individuals. *Journal of Science and Medicine in Sport*. **13** (1) 74–79 (2010).
28. Midgley, A.W., McNaughton, L.R. Jones, A.M. Training to enhance the physiological determinants of long-distance running performance: can valid recommendations be given to runners and coaches based on current scientific knowledge? *Sports Medicine*. **37** (10) 857–880 (2007).
29. Jones, A.M. DiMenna, F.J. Cardiovascular Assessment and Aerobic Training Prescription. In: Strength and Conditioning: Biological Principles and Practical Applications. M. Cardinale, R. Newton and K. Nosaka, eds. London: John Wiley & Sons, Ltd. 291–304 (2011).
30. Beneke, R. von Duvillard, S.P. Determination of maximal lactate steady state response in selected sports events. *Medicine and Science in Sports and Exercise*. **28** (2) 241–246 (1996).
31. Beneke, R. M., Hütler, M. Leithäuser, R.M. Maximal lactate-steady-state independent of performance. *Medicine and Science in Sports and Exercise*. **32** (6) 1135–1139 (2000).
32. Smith, C. G. Jones, A.M. The relationship between critical velocity, maximal lactate steady-state velocity and lactate turnpoint velocity in runners. *European Journal of Applied Physiology*. **85** (1–2) 19–26 (2001).
33. Pringle, J. S. Jones, A.M. Maximal lactate steady state, critical power and EMG during cycling. *European Journal of Applied Physiology*. **88** (3) 214–226 (2002).
34. Mattioni Maturana, F., Keir, D.A., McLay, K.M. Murias, J.M. Can measures of critical power precisely estimate the maximal metabolic steady-state? *Applied Physiology Nutrition and Metabolism*. **41** (11) 1197–1203 (2013).
35. Jones, A.M, Burnley, M., Black, M.I., Poole, D.C. Vanhatalo, A. The maximal metabolic steady state: redefining the 'gold standard'. *Physiological Reports*. **7** (10) e14098 (2018).
36. Scheuermann, B.W. Kowalchuk, J.M. Attenuated respiratory compensation during rapidly incremented ramp exercise. *Respiratory Physiology*. **114** (3) 227–238 (1998).
37. Morgan, D.W., et al. Variation in the aerobic demand of running among trained and untrained subjects. *Medicine and Science in Sports and Exercise*. **27** (3) 404–409 (1995).

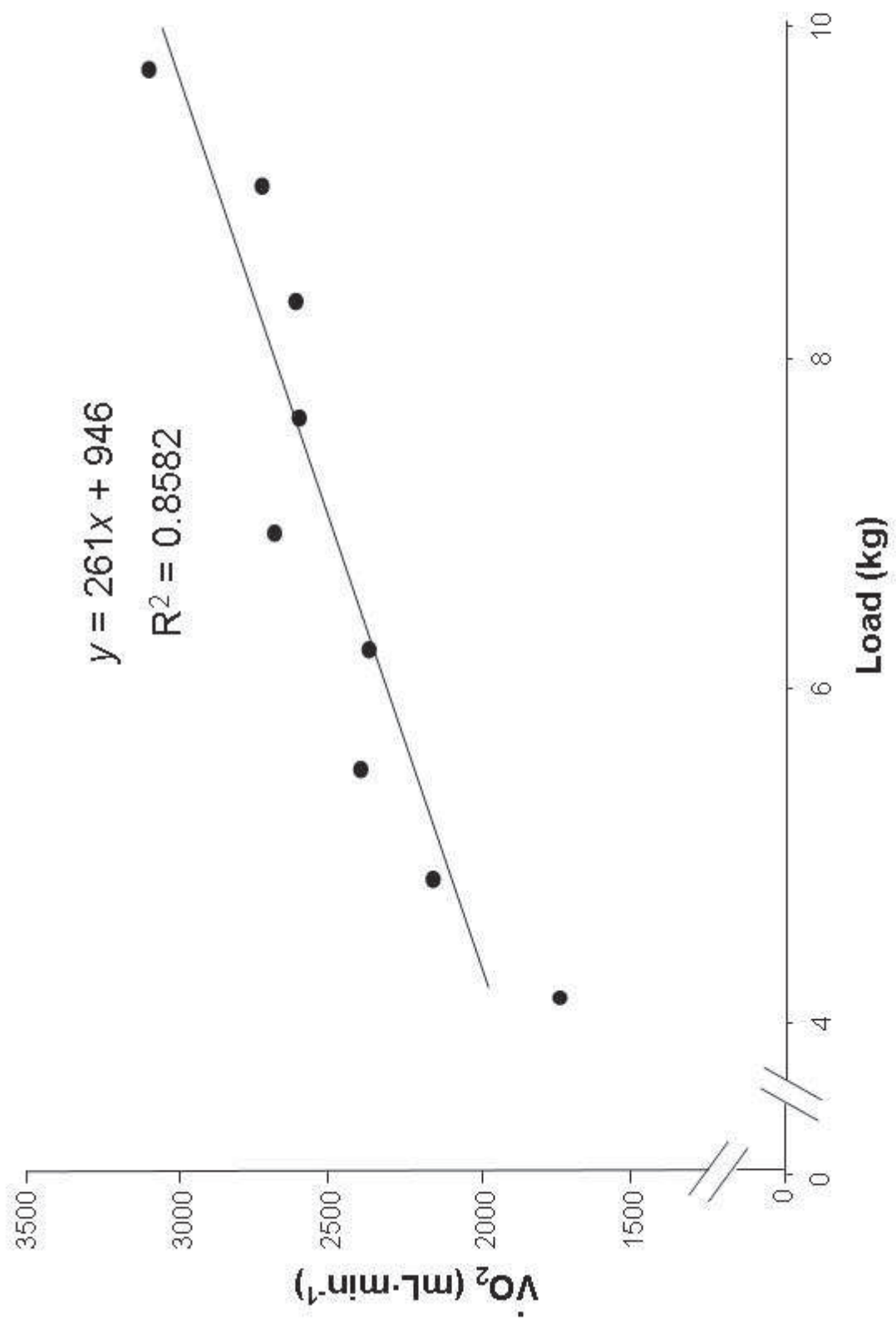
- 656 38. Franch, J., Madsen, K., Djurhuus, M.S. Pedersen, P.K. Improved running economy
657 following intensified training correlates with reduced ventilatory demands. *Medicine and*
658 *Science in Sports and Exercise*. **30** (8) 1250–1256 (1998).
- 659 39. Holmer, I., Lundin, A. Eriksson, B. Maximum oxygen uptake during swimming and
660 running by elite swimmers. *Journal of Applied Physiology*. **36** (6) 711–714 (1974).
- 661 40. Bonen, A., Wilson, B.A., Yarkony, M. Belcastro, A.N. Maximal oxygen uptake during free,
662 tethered, and flume swimming. *Journal of Applied Physiology: Respiratory, Environmental and*
663 *Exercise Physiology*. **48** (2) 232–235 (1980).
- 664 41. Magel, J.R. Faulkner, J.A. Maximum oxygen uptakes of college swimmers. *Journal of*
665 *Applied Physiology*. **22** (5) 929–933 (1967).
- 666 42. Poole, D.C. Jones, A.M. Measurement of the maximum oxygen uptake $\dot{V}O_{2\max}$:
667 $\dot{V}O_{2\text{peak}}$ is no longer acceptable. *Journal of Applied Physiology*. **122** (4) 997–1002 (2017).

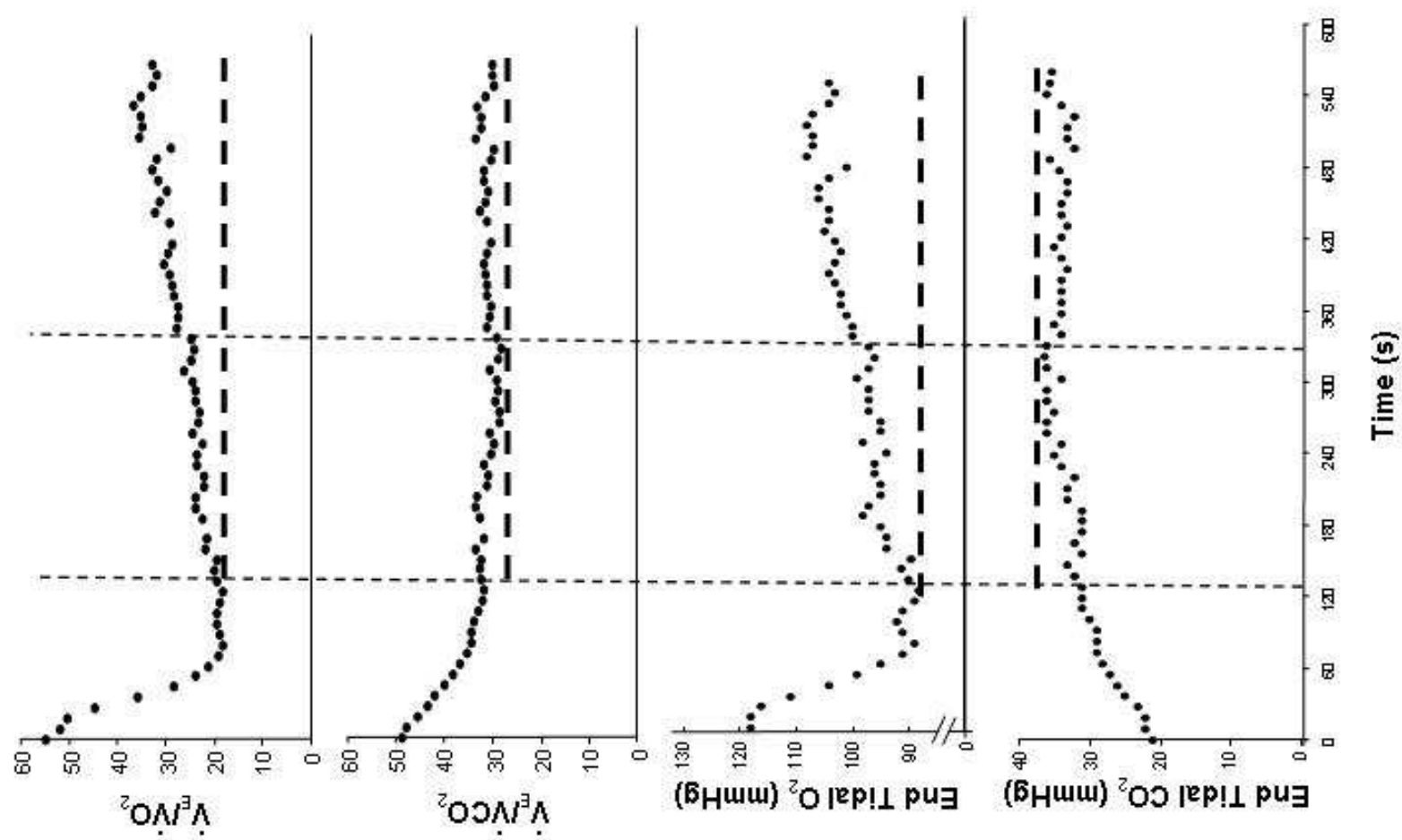


Figure

[Click here to access/download;Figure;Figure 2-1.eps](#)







Body mass (kg)	84.7
Stature (cm)	184
Time to limit of exercise tolerance (s)	576
Stages completed	9.6
Peak load (kg)	10.5
$\dot{V}O_{2\text{peak}}$ (L·min ⁻¹)	3.44
$\dot{V}O_{2\text{peak}}$ (mL·min ⁻¹ ·kg ⁻¹ BM)	40.6
$\dot{V}O_{2\text{GET}}$ (L·min ⁻¹)	2.57
$\dot{V}O_{2\text{GET}}$ (mL·min ⁻¹ ·kg ⁻¹ BM)	30.3
$\dot{V}O_{2\text{GET}}$ (% $\dot{V}O_{2\text{peak}}$)	75
$\dot{V}O_{2\text{RCP}}$ (L·min ⁻¹)	2.95
$\dot{V}O_{2\text{RCP}}$ (mL·min ⁻¹ ·kg ⁻¹ BM)	34.8
$\dot{V}O_{2\text{RCP}}$ (% $\dot{V}O_{2\text{peak}}$)	86
$\dot{V}O_2$ -load slope (mL·min ⁻¹ ·kg ⁻¹)	261
$\dot{V}O_2$ -load slope (mL·min ⁻¹ ·kg ⁻¹ BM·kg ⁻¹)	3.1

Name of Material/ Equipment	Company
3-L syringe	Hans Rudolph
Aquatrainer	COSMED
K4b2	COSMED
N200PRO	Cefise
Pacer 2 Swim	Kulzer TEC
Tether-system	Own design
Tether attachment	CEFISE

Comments/Description

Calibration device

Snorkel system/gas-exchange measurement

Portable CPET unit/gas-exchange measurement

Software program for analysis of force signal

Swimming velocity management/underwater LED line

Pulley-Rope system/loading management

Bracket for attachment to swimmer

Dear Dr. Dr. DiMenna,

Your manuscript, JoVE60630 "Maximal Incremental Exercise Testing for Cardiorespiratory Assessment of Swimmers: A Rapidly-incremented Tethered-swimming Protocol," has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.

After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps., .svg). Please ensure that the image is 1920 x 1080 pixels or 300 dpi. Additionally, please upload tables as .xlsx files.

Your revision is due by Sep 19, 2019.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision". Please note that the corresponding author in Editorial Manager refers to the point of contact during the review and production of the video article.

Best,

Phillip Steindel, Ph.D.
Review Editor
JoVE
617.674.1888
Follow us: [Facebook](#) | [Twitter](#) | [LinkedIn](#)
[About JoVE](#)

*Dear Dr. Steindel,
Thank you for affording us the opportunity to submit a revised version of our manuscript.
We have attempted to address each of your comments as well as those of the reviewers.
We welcome any additional feedback that you can offer to further improve the manuscript.*

Editorial comments:

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have proofread the manuscript for any spelling or grammar issues that were present.

2. Please rewrite your title to avoid the subtitle.

We have rewritten the title according to your suggestion.

3. Please ensure you use 'mL' instead of 'ml', including in the figures.

We have changed "ml" to "mL" throughout the manuscript and on Figure 3.

Protocol:

1. For each protocol step, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

We apologize for our inability to get this aspect of the article correct. We have never done an article like this before. Hopefully, our protocol steps are now more complete. If it is still insufficient, please let us know.

Specific Protocol steps:

1. Please rewrite the instructions to swimmers in the beginning of the protocol as numbered steps in the imperative.

We have made this change.

2. 1.3.7-1.3.8: Is this trial 30 s as well?

We have clarified this.

3. 2.2.5-2.2.8: How are these calibrations done? Are these standard procedures for the instrumentation used?

Yes, this is standard procedure. Depending on the program, you simply click a button that initiates the process. The unit will sample both ambient air and the gas samples you provide with the specific concentrations that serve as the reference values. This was explained in a previous JoVE article so we have added that in as a reference.

Figures:

1. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account.

We have uploaded an email exchange that I had with Aleksandra Mostowik from the Editorial Office of Journal of Human Kinetics. Please let me know if this is not sufficient and why because if so, I will obtain whatever is needed.

Table of Materials:

1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

Thank you for this suggestion. We have double checked and found one item that was indeed missing. I believe we have everything covered now.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The evaluated work is of good scientific and technical level. Advances in knowledge in the area by proposing a simpler and faster test for measuring oxygen consumption of swimmers.

Dear Reviewer #1,

Thank you for taking the time to review our manuscript. We are glad to learn that you found our work of good scientific and technical level. We have addressed each of your concerns and look forward to any additional feedback you might be able to provide.

Major Concerns:

The abstract could describe some data obtained from the protocol.

We have added information regarding the metabolic rates at GET and RCP for our representative subject to the Abstract. Unfortunately, the 300-word limit restricts us from adding anything more, but these are at least the most important data that this test reveals.

Minor Concerns:

62/5000

Why is the text marked in yellow from line 170?

In section C of the "Instructions for Authors" for this journal under the heading of "Protocol length and highlighting," bullet-point #2 states: "For a protocol section that exceeds 3 pages, highlight in yellow up to 2.75 pages (no less than 1 page) of protocol text (including headers and spacing) to be featured in the video. Our scriptwriters will derive the video script directly from the highlighted text."

Reviewer #2:

Manuscript Summary:

In this paper, the authors provide methodological considerations regarding a novel method to assess parameters of aerobic function (VO₂max, GET, and RCP) during a

rapidly-incremented ramp-test for swimming. The manuscript is well written and it provides accurate details on how to conduct and interpret such a test. I have few comments for the authors.

Dear Reviewer#2,

Thank you for taking the time to review our manuscript. We appreciate your detailed comments and have acted upon each one. We welcome the opportunity to address any lingering concerns you might have with our revised version.

Comments:

Line 79: "Maximal" relative to the ramp-slope of use

We have added this qualifying statement.

Line 81: "achieve" better than "endure" here.

Change made.

Line 83: I see that the authors are distinguishing between VO₂peak and VO₂max. I wouldn't say "based on the assumption that it is the most rapid rate at which the individual can consume O₂ per se". In the majority of cases the highest VO₂ recorded at the end of a RI test is the same - if not higher - than the one recorded during, for instance, a time-to-exhaustion trial. Thus, I would say that generally a RI test provides the highest rate at which O₂ can be consumed. After all, this was also the original and correct interpretation of Whipp (1981).

We have removed that parenthetical addendum.

Line 87: I would say "non-invasive and indirect"

Addition made.

Line 91: although the occurrence of LT has solidly been linked to lactate changes within the blood, the authors should be careful when linking the RCP with an irreversible accumulation of lactate. The RCP is a complex phenomenon that, although strictly linked to the increasing contribution of the "anaerobic pathway" to the energy demand, originates because of many different factors.

We have qualified this statement.

Lines 79-91: although the observations described in this paragraph are well established, it would be good to provide some references. After all, they are all based on other people's work...

We have added references to support the statements we have made in that section.

Line 100: another pertinent reference here is Iannetta et al., SJMSS 2018.

We have added that reference.

Line 102: as the authors surely know, although the VO₂ between the RCP and CP/MLSS are in most instances the same, the WR is not. Therefore, I would add something like this: "although attention should be paid when translating ramp-derived WR to CWR" (Keir et al., APNM 2018; DiMenna et al., JESF 2009).

We have added a statement to clarify this.

Line 119: This is something that I disagree with. The slope of the VO₂/WR relationship cannot be used to calculate delta-efficiency, because it is affected by the kinetic property of the muscle pools being recruited. A greater slope not necessarily means a lower efficiency. Vice-versa a smaller slope not necessarily means an improved efficiency (Keir et al., JAP 2016; Boone et al., Sport Medicine 2012).

We appreciate the concerns you have voiced about this here and below. One option to address these concerns would be for us to remove all mentions of the VO₂-work rate slope that can be derived from this test based on the contention that it has no relevance as a measure of exercise economy. If you believe that this is warranted, we will do so; however, we would rather not because: 1.) we believe that the parameter does have relevance in this regard with appropriate interpretation; and 2.) the objective of this article was to provide instructional video regarding a test that we have previously documented in a published paper that did indeed include mention of derivation of the VO₂-work rate slope from this protocol (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5504584/>). If readers/viewers are doing so to derive more information about implementing the test they have read about previously, there will not be congruence between the two publications if we remove it completely. With these two things in mind, we have chosen to leave it in and qualify the statements about its usefulness. We welcome your feedback regarding any additional information we can include for this purpose or, alternatively, if you believe all references to this parameter should be removed.

Line 157: how many participants?

We have added this information.

Line 282: bike tests usually do not have a warm-up phase. This practice can alter the gas-exchange profiles by modulating the CO₂ stores at ramp-onset. Furthermore, I see that the pace is not controlled (what is moderate in this case?). Thus, this could potentially affect the reproducibility of the GET and the slope of VO₂ increase. Don't the authors think that this phase should be better controlled? Or avoided?

Unlike cycling, swimming requires acute accommodation to both a more complex movement pattern and also performing the movement in the water environment. These were competitive swimmers and they were instructed to swim at a pace that was not taxing. This is something they are well accustomed to in the competitive arena. We have changed “moderate” to “light” which is a better description and emphasised that this should just be done with care taken to avoid engendering any lingering effects that could influence the results of the test.

Line 427-429: this would be a good place to cite a recent paper from Iannetta et al. (MSSE 2019).

Citation added.

Line 430: "non-linearity for a given person". I'm not sure this is clear. Consider rephrasing, perhaps referring specifically to the non-linear behavior of VO₂ across the intensity spectrum.

Rephrased.

Line 440-441: it is important to mention here "above that which would be predicted by linear extrapolation of the VO₂ cost from the moderate-domain".

Added.

Line 445: not necessarily VO₂ attains VO₂max. Exhaustion can occur earlier. I would mention that in most cases, but not all, VO₂max is attained.

We have qualified this statement.

Lines 448-450: Well, although agreeing with the authors on the fact that the intensity domain schema currently provides the best strategy to normalize exercise intensity, I have some doubts about the fact that this schema is well accepted in training settings. Uncountable exercise training zones exist out there which have little to do with physiological boundaries and responses. No need to change anything; this is just a personal consideration.

Agreed. What we meant was that training at different intensities on different days of a training regimen is well accepted. Unfortunately, determination of those varied intensities is often done haphazardly as you suggest. Hopefully, as we continue to spread the word, this will change.

Lines 459-471: First of all, why do the authors feel the need to discuss the equivalence between MLSS and CP in this paper? Second, the authors basically affirm that CP is superior to MLSS in detecting the heavy-to-severe boundary. In support to this supposition, they list several reasons discrediting MLSS. The result is that the reader is pushed to think that MLSS is flawed while CP is perfect. Indeed, the issues with the CP

estimation are not mentioned at all. This is important, because the papers cited to support the lower WR at MLSS compared to CP used different methods and approaches for CP estimation. Thus, my question to the authors is: can we undoubtedly say that MLSS underestimates CP if this observation is based on studies that have used different methods to estimate CP? Furthermore, as the authors partly point out, the accuracy of MLSS is based on the delta change used from one trial to another. Again, the studies cited (and others) used different delta changes. Therefore, is it fair to conclude what the authors are concluding without acknowledging all the issues associated with MLSS and CP estimations? What I'm trying to say is that both methods (MLSS and CP) give very good estimations (when measured with gold standard procedures) of the heavy-to-severe boundary. Each of them have pros and cons. These, I believe, need to be acknowledged when discussing their equivalence.

There really is no need (or ability) for us to enter into that controversy in this paper so we have changed that section to simply state that both methods can be used but they do not always provide the same estimate. If the reader is interested in learning more about this, they can access the references that have been provided.

Lines 475-477: What do the authors imply with this statement?

Statement removed (see previous comment).

Lines 485: this paragraph finds me is disagreement. Exercise economy cannot be derived from rapidly-incremented exercise test, for the reason that I have explained before.

See previous comment about this.

Lines 496-513: By looking at figure 2 it seems that the rate of increase is $\dot{V}O_2$ is not constant throughout the incremental-test. Don't the authors think that increasing load may alter the force dynamics applied in the water and change the $\dot{V}O_2$ cost in a non-linear way? These potential biomechanical changes should be at least mentioned. This is also why I don't think the slope of $\dot{V}O_2$ can be used to calculate efficiency, because this slope will be affected not only by the kinetic properties, but also by the way each swimmers adapts from a biomechanical perspective to the increased "load". This might explain the variability seen in Figure 3.

We have added an explanation about this to the paragraph in the Discussion within which we speak about how increasing load during tethered swimming in an incremental manner might result in different adaptations to the increase of intensity compared to increased velocity during free swimming.

Figure 4. I see in figure 4 that the breakpoints (especially the RCP) are not very marked. This is somewhat different from what is typically observed when using running or bicycle ergometers. I think the authors should comment on this. How does this affect the identification of these breakpoints?

Any physiological reason (muscle mass? Breathing patterns?)?

We have made mention of this in the Introduction.

Also values for end tidal pressures of O₂ and CO₂ should be expressed in absolute units (not percentages).

We have changed the units on Figure 4.

DiMenna, Frederick <DiMenna@exchange.tc.columbia.edu> Wed, Jul 15, 10:00 AM

to Dalton, Journal

Dear Aleksandra,

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Sincerely,
Fred

Journal of Human Kinetics <jhk@awf.katowice.pl> Wed, Jul 17, 4:32 AM

to Frederick

Dear Fred,

Thank you for contacting me and congratulations on the opportunity you mentioned. Absolutely you may use the Figure from the paper you published in JHK. It would be nice if you could provide some reference if there is such a possibility, that's all.

Have a good day and remember about us when choosing a Journal for your future submission ☺
All the best,

Aleksandra Mostowik
Editorial Office of Journal of Human Kinetics
Academy of Physical Education in Katowice
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Title of Article:	Maximal Incremental Exercise Testing for Cardiorespiratory Assessment of Swimmers: A Rapidly-incremented Tethered-swimming Protocol
Author(s):	Pessoa Filho, Massini, Siqueira, Santos, Vasconcelos, Almeida, Espada, Reis, Alves, DiMenna

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