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LETTER TO THE EDITOR

Time course of biochemical variables and comparisons between internal and external load responses in tethered swimming

Variations des constantes biochimiques et comparaisons entre les réponses en charge interne et externe dans la nage en résistance

1. Introduction

The aerobic metabolism is predominant in swimming events above 100 m, indicating that middle and long-distance swimmers should improve the effectiveness of this pathway during their training routines. In addition, the aerobic metabolism has an important role during recovery between consecutive high-intensity efforts, removing metabolites and promoting high-energy phosphate resynthesizes, which are essential factors to maintain performance of subsequent bouts, as often observed in high intensity swimming training. Thus, improving aerobic fitness is also important for sprinters, allowing intensity maintenance during sessions.

Maglischo [1] divided aerobic stimuli into three categories, basic endurance training (End-1), threshold endurance training (End-2), and overload endurance training (End-3). End-1 is performed below the anaerobic threshold intensity (AnT) with high-volume, being used as a regenerative stimulus and to maintain aerobic adaptations [1]. Differently, End-2 is performed at AnT, developing swimmer aerobic capacity [1]. End-3 sessions use intensities above AnT, near maximal aerobic intensity [1], in which maximal oxygen consumption is achieved and aerobic power can be improved. These stimuli are applied in free-swimming, allowing the training sessions and time expended to cover a specific distance to be monitored.

Although the use of time is very helpful during training routines, some factors can influence the global intensity of the session. First, the session can be performed at high intensity and have an important role during competition. Some swimmers can increase the submerged phase after turns, decreasing the number of strokes and, consequently, lowering the time expended for a given intensity during the session. In addition, considering that AnT determination is protocol-dependent, and the effort-recovery ratio influences physiological responses, some adjustments are necessary to prescribe sessions using different distances, which should be further investigated. Thus, although the most specific form of training is free-swimming efforts, they can require different physiological aspects with the same time to cover a prescribed distance, which could influence the adaptation expected from using different aerobic stimuli. Alternatively, tethered swimming can be used, allowing also continuous efforts demonstrating two main advantages: first, prescription at the exact intensity related to aerobic parameters (e.g., AnT) [2] and time of force application in each stroke increases due to a longer propulsion phase, which improves force maintenance during middle/long distance events. In addition, tethered swimming can be applied to train teams [2]. Although some alterations in swimming mechanics have been observed, a recent study showed no change in stroke parameters after seven weeks of tethered swimming using elastic cord during typical training sessions [2]. Furthermore, peak blood lactate concentrations ($[La^-]$) are higher in tethered swimming training [2]. Although is not a substitute for traditional free-swimming stimuli, the use of tethered swimming could be an interesting alternative during the training routine.

Change the level of catabolism can happen after sessions using tethered swimming. In addition, uric acid is an important antioxidant and a discrete injury marker, following acute short-term maximal exercise, in which levels are increased and can be further enhanced by lactate-induced inhibition of renal clearance. In this context, although several methods are valid to monitor the internal training load (ITL) in free-swimming (e.g., ratings of perceived exertion (RPE) and blood lactate concentrations ($[La^-]$), specific characteristics of tethered swimming may influence the relations with external training load (ETL), compromising its use during a training routine.

If these hypotheses are correct, the inclusion of tethered swimming sessions during a training routine, time course of biochemical variables related to muscle damage, catabolism, and UA values should be investigated. Additionally, the relationships between ETL (i.e., force during

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efforts) and ITL markers (e.g., RPE and [La^-]), should also be tested. Thus, the objectives of the present study were to:

- test the relationships between ETL and ITL in tethered swimming;
- investigate acute responses and time course during recovery of biochemical variables, in three different aerobic sessions (i.e., End-1, End-2, and End-3) performed in tethered swimming;
- verify the ability (i.e., sensitivity) of the variables to distinguish End-1, End-2, and End-3.

2. Equipment and methods

2.1. Participants

Five males (15.00 ± 1.58 years; 160.50 ± 9.98 cm; 60.07 ± 13.31 kg; body fat: $10.96 \pm 7.25\%$) and six females (15.38 ± 1.18 years; 162.38 ± 5.15 cm; 60.41 ± 7.54 kg; body fat: $20.01 \pm 5.07\%$), voluntarily participated in the current study. The swimmers had at least two years' experience at state and national level, performance was 152.97 s for the 200 m distance in competitions, training six times a week with an average volume of 8.000 m.day^{-1}. Tethered swimming efforts were common during the competitive phase of training for these swimmers, in addition, all tests performed were part of the training routine for at least one year. All procedures were approved by the Institutional Review Board for Human Subjects of the University (Human Research Ethics Committee, n°187/2013). Athletes and their parents or legal guardians were informed about the experimental procedures and provided a written informed consent form authorizing the swimmers' participation in the study. The experimental protocol was approved by the local Ethics Committee and conformed to the principles outlined in the Declaration of Helsinki.

2.2. Experimental approach to the problem

First, AnT and the maximum aerobic force (MAF) were determined through a graded exercise test in tethered swimming. Subsequently, swimmers performed tethered swimming sessions at End-1, End-2, and End-3. All tests and sessions were separated by 72 h of recovery. During the sessions, RPE and [La^-] were determined at baseline, middle (50% of the volume), and immediately after the bout (100% of the volume). Blood samples to determine biochemical variables (i.e., muscle damage, catabolism, and UA) were collected in the morning and fasted athletes before baseline, and after 24 hours of recovery. Blood samples too going collected immediately after the effort (0 h).

2.3. Instrumentation and blood samples

All forces were measured using a dynamometer (HOMIS 2100, São Paulo - Brazil), equipped with a load cell (100 kg capacity) as the primary sensor. Athletes were connected to the force system through a six-meter long elastic cord (Auriflex n°204, São Paulo, Brazil) attached to a belt around their waist. The other end of the elastic cord was connected to the load cell (CSR-100 kg, MK Controle®, São Paulo, Brazil). Data from the load cell were registered every two seconds and recorded using specific software (Lutron SW-U801, Taipei-Taiwan). First, elastic cord was calibrated through the superposition of known weights, this process of calibration used during of tests, was previously by our laboratory [2]

Blood samples (25 μL) were collected from the ear lobe for [La^-] determination (Yellow Springs Instruments model 1500 Sport, Ohio, USA). Biochemical variables were assessed by 20 ml of blood from the right antecubital vein using a vacuum collection method.

2.4. Tethered swimming graded exercise test and aerobic session prescription

The graded exercise test was performed until voluntary exhaustion or until the swimmer was unable to sustain a predetermined force for more than 10 s. Initial intensity was 20 N, with increments of 10 N every three minutes. Exercise intensities and increments were measured with a mark on the bottom of the pool, allowing the swimmers to maintain the proper position and force. Real force exerted during each stage was defined by mean force exerted during last minute of stage. Blood samples were collected to determine [La^-] after each stage (≅ 30 s of break).

If the swimmer could not maintain force before finishing the stage, peak force exerted during the graded exercise test was adapted for tethered swimming [2]. AnT was assumed as the swimming intensity corresponding the intersection of two straight lines. The points, acquired from the relationship between [La^-] and force (i.e., bi-segmented model) [2]. This method is valid to determine maximal lactate steady state (i.e., gold standard for the assessment of aerobic capacity).

The End-1 session lasted 45.45 ± 5.22 min, below AnT intensity (e.g., 75—90% of AnT), this session was composed of continuous efforts. The End-2 session was performed for 30 min at AnT intensity and 6 efforts were performed with a 5 min duration. The End-3 session lasted 20 min, performed through 10 bouts of 2 min at 110—150% of AnT. Passive rest periods were allowed between each effort (≅ 30 s of break) for both End-2 and End-3.

Mean force (MF) during exertion was determined as the average of the final 30 s of each effort. In addition, [La^-] and RPE (i.e., 6—20 point scale) were collected at the end of each session (all participants train with use of the scale for at least one year). Thus, the ETL of different sessions was considered as the product between MF and total duration of the session. ITL was determined using both [La^-] (i.e., ITL = [La^-]·duration; ITL-[La^-]) and RPE [i.e., ITL = RPE·duration (s); ITL-RPE both expressed in arbitrary units (a.u.)

2.5. Biochemical Variables

Muscle damage was assessed through concentrations of lactate dehydrogenase (LDH) and creatine kinase (CK) enzymes. [CK] was assessed using an ''MPR3 CK NAC-activated'' kit (Boehringer Mannheim), buffer solution (2.5 mL) and a specific reactive tablet for exposure to the

Table 1 Acute responses and training load relative to aerobic sessions prescribed below AnT (End-1) at anaerobic threshold (End-2), and above AnT (End-3).

	End-1	End-2	End-3
Session responses			
MF (N)	34.6 ± 10.8[a]	42.3 ± 11.1[a]	51.9 ± 9.6[a]
[La⁻] (mmol/l)	1.3 ± 0.4	2.4 ± 1.7[a]	5.2 ± 3.1[a]
RPE (a.u.)	9.8 ± 2.5[a]	12.9 ± 2.1[a]	15.9 ± 1.8[a]
Session load			
ETL (a.u.)	1578,1 ± 648.8[a]	1917.9 ± 680.2[a]	2314.1 ± 544.4[a]
ITL-[La⁻] (a.u.)	58.0 ± 24.7	72.0 ± 51.8	120.3 ± 56.9[a]
ITL-RPE (a.u.)	446.0 ± 156.5	385.5 ± 62.9	386.2 ± 81.0

MF: mean force; [La⁻]: blood lactate concentrations; RPE: rate of perceived exertion; ETL: external training load; ITL-[La⁻]: internal training load determined through [La⁻]; ITL-RPE: internal training load determined through RPE.
[a] significant differences from other two sessions ($P < 0.05$).

water bath (37 °C) until complete tablet dissolution. Next, 50 μl of plasma was added to the reactive solution, again being left in a water bath (37 °C) for one minute.

Immediately after, four absorbance readings of a 334 nm sample were performed, with a time interval between readings, to obtain the Δ value. The CK commonly used clinical units (U/L) activity was calculated as CK = 8252 × Δ absorbance/minute. The UV kinetic method (Advia kit, Bayer, USA) was used to determine LDH activity. The reagents and samples were exposed at room temperature, a temperature of 37 °C, and wavelength of 340 nm, using a cuvette with a 1 cm light passage.

Catabolism induced by different sessions was evaluated by blood cortisol concentrations, determined through the chemiluminescence method, IMMULITE, DPC med. lab, using solid phase kits with labeled antibodies.

Analyzes for UA concentration in plasma were conducted using the colorimetric method (enzymatic trinder). Samples were homogenized and placed in a 37 °C water bath for five minutes. Sample and standard absorbance were determined at 505 nm (490—540 nm), and zero was corrected for white. The color is stable for 30 min. The calculation for uric acid determination (mg/dL) is (Sample Absorbance/Standard Absorbance) × Standard Concentration (mg/dL).

2.6. Statistical analysis

Data normality was tested by the Shapiro Wilk test, allowing the data to be shown as means and standard deviations. Sphericity was verified through Mauchly's test and corrected, when necessary, using the Greenhouse-Geiser test, allowing the use of parametric statistics. Analysis of variance for repeated measurements, followed by the Bonferroni post-hoc was used to compare training loads in different sessions. In addition, possible correlations between ETL and ITL indices (i.e., ITL-[La⁻] and ITL-RPE) were tested by Pearson's correlation.

The comparison between biochemical variables evidenced in different training sessions and during different moments was performed by mixed model analysis with fixed factors (i.e., 'aerobic session' and 'time of analysis') and both repeated and random factor (i.e., subject). Statistical Package for Social Science software, version 17.0 (SPSS Inc, Chicago, Illinois) was used and the level of significance was set at $P < 0.05$.

3. Results

Average AnT presented a value of 42.74 ± 10.21 N. Table 1 demonstrates the MF, [La⁻], and RPE observed after three aerobic sessions. MF and RPE significantly increased between End-1, End-2, and End-3 ($P < 0.001$). However, no [La⁻] differences were observed between End-1 and End-2 ($P = 0.20$). Although ETL presented a significant increase between sessions ($P < 0.004$), no ITL-[La⁻] differences were observed for End-1 and End-2 ($P = 0.66$). In addition, ITL-RPE was not sensitive to differentiate aerobic sessions ($P < 0.70$). ITL-[La⁻] presented significant correlations with ETL after End-1 ($r = 0.87$; $p = 0.001$), which did not occur in other sessions (End-2: $r = -0.06$; $P = 0.65$ and End-3: $r = -0.34$; $P = 0.87$). ITL-RPE was not related with ETL (End-1: r = 0.57; $P = 0.07$; End-2: $r = 0.33$; $P = 0.33$ and End-3: $r = 0.13$; $P = 0.70$). ITL-RPE was related with ITL-[La⁻] only at End-1 ($r = 0.80$; $P = 0.01$), which did not occur in other sessions ($r < -0.56$; $P < 0.03$).

Fig. 1 demonstrates the [LDH] and [CK] responses at baseline and recovery periods. No effects of aerobic session or time of sample were observed for [LDH] ($P = 0.12$) or [CK] ($P = 0.46$).

For cortisol concentrations, an interaction between sessions and moment was observed ($P = 0.02$), indicating that End-3 induced higher concentrations at 0 h in relation to other sessions ($P < 0.01$) (Fig. 2). No differences were observed in UA responses ($P = 0.68$).

4. Discussion

The main results were:

- although ETL presented a significant increase in different training sessions in tethered swimming, no differences were observed for ITL obtained through RPE; the insensitivity of ITL-[La⁻] (for End-1 and End-2) and ITL-RPE (for End-1, End-2, and End-3) to distinguish different sessions of training;
- only End-3 generated significant increases in [La⁻];

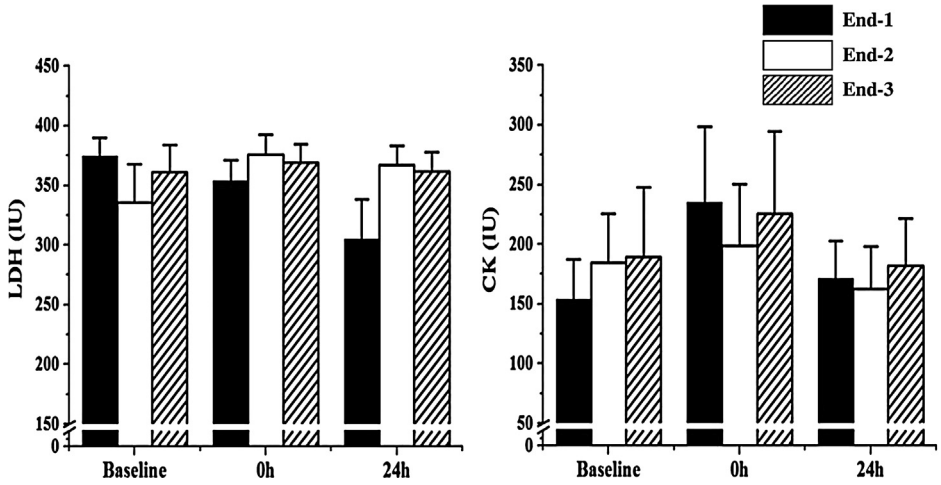

Figure 1 Results of lactate dehydrogenase (LDH) and creatine kinase (CK) in the situation basic endurance training (End-1), threshold endurance training (End-2), and overload endurance training (End-3) in the different time baseline, 0 h and 24 h after the efforts.

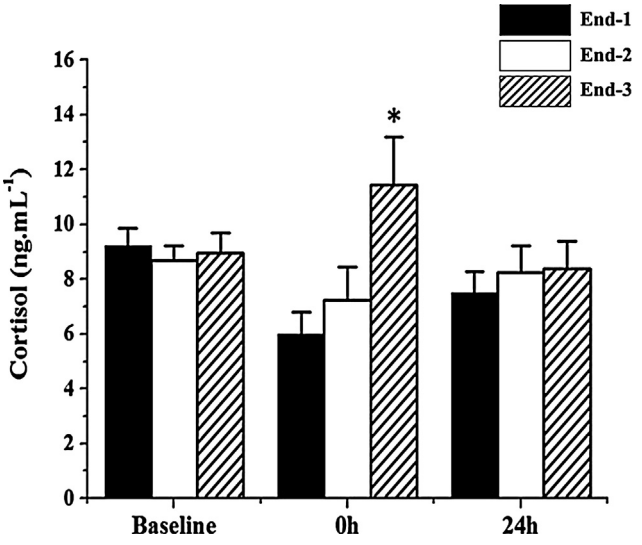

Figure 2 Cortisol concentrations in different sessions and moment (baseline, 0 h and 24 h after effort). Basic endurance training (End-1), threshold endurance training (End-2), and overload endurance training (End-3) in the different time baseline, 0 h and 24 h after the efforts.*different from all moment in the session.

- only cortisol showed sensitivity to different levels of intensity. However, there were no differences between pre and post-training situations, demonstrating that, with respect to the biochemical characteristics, whatever performing exercise acute below, the intensity or above Ant.

The division of training into three different intensities was systematized by Maglischo et al. [1] for free swimming. As evidenced by Papoti et al. [2], tethered swimming presents advantages, which favor its insertion in training routines (e.g., sensitivity to training responses similar to free swimming). In addition possibility of prescription in exact intensity [2] and increases the time of application of force during stroke [1]. However, to our knowledge, this is first study to investigate acute responses (i.e., biochemical parameters) in different training intensities of tethered swimming, evidencing important results on swimming periodization as well as the insertion of this model during training.

The use of RPE to quantify training loads has shown high correlation with other methods, such as oxygen consumption and heart rate. In addition, RPE has been validated with methods that used [La^-], ventilatory variables, and HR responses [3]. However, in the present investigation, ITL-RPE was not sensitive to different aerobic training sessions in tethered swimming. Furthermore, it did not present correlations with ETL, corroborating with other studies. In swimming, correlations were found between ITL (i.e., RPE) and ETL (i.e., distance), however, this is the first study to verify correlations between ETL and ITL in tethered swimming sessions, requiring further investigation to broaden the understanding of this relation.

In training sessions below and at AnT intensity, [La^-] tends to not to change significantly. During exercise performed above AnT, [La^-] tends to increase over time, corroborating with our results. In the literature have showed that the intensity at AnT (i.e., End-2) [La^-] tends to be lower (i.e., $\approx$3 mmol/l) (i.e., $\approx$4 mmol/l). This fact explains why End-1 and End-2 did not present significant changes in relation to [La^-]. Additionally, during End-1 and End-2 sessions, the upper body segments are responsible for the majority of the propulsive force [4], while End-3 demands greater participation of propulsion from the legs, which increases oxygen consumption and, consequently, [La^-], supporting the findings of the present study.

Changes in training volume and intensity result in an increase in cortisol concentrations and it has been suggested that this hormone be monitored to verify negative training adaptations and overreaching/overtraining. High intensity-short duration resistance exercise causes an increase in cortisol concentrations, however, an acute increase has no relation to protein catabolism, since high-intensity resistance exercises stimulate protein anabolism and skeletal muscle hypertrophy, confirming our findings, since corti-

sol levels returned to basal values after 24 hours. Moderate intensities presented no changes in cortisol concentrations, corroborating with other studies, which demonstrate that after 24 h, athletes would be prepared for further efforts independent of the intensity.

The levels of [CK] are elevated during exercise to maintain muscle ATP, this being used as a marker of damage due to physical stress. In swimmers, changes in volume and intensity cause [CK] alteration. Mougios [5] proposed that swimmers have lower concentrations than other modalities (e.g., soccer) mainly because of the form of training. Regardless of training intensity, [CK] presented similar values, not reaching the upper limit (i.e., 523 U/L) proposed by Mougios [5]. Moreover, after 24 h, no significant differences were observed. Acutely, [CK] was independent of intensity, and the cumulative effect of efforts may be responsible for changes in [CK].

As well as CK, LDH is also used as a marker of muscle damage since an increase is observed after high intensity exercises. The present study agrees with other authors who did not observe changes in [LDH] after a training session at medium intensity. Other authors also did not find alterations in this marker after a week of training and [CK] presented similar behavior in water polo players.

In literature, there has been an increase in [UA] is observed after anaerobic and aerobic exercises. In the literature have found that [UA] is affected by swimming intensity, however there were no significant differences between different training models. High [La^-] also alters UA elimination. Papoti et al. [2] demonstrated that tethered swimming is able to improve lactate production, although a training session may not have been enough to elevate [La^-] to the point of altering UA elimination.

The chronic effects of these physiological and biochemical variables 48 h post-effort were not measured. Although this could be considered as a limiting factor, we aimed to verify if these parameters would return to normal after 24 h which would allow the athletes to carry out a new training session. In addition, the mixed sample may increase the heterogeneity of the group. A possible important limitation is assessing RPE immediately after the session, when it is suggested that RPE is measured 30 min after a session, which could influence training load magnitude using this variable.

As a practical application Papoti et al. [2] demonstrated that tethered swimming can be used to evaluate training responses and prescribe specific training sessions for young swimmers. However, due to increased force application time and by allowing the use of continuous longer efforts, tethered swimming training can induce higher levels of muscle damage [1]. In addition, the possibility of controlled the intensity of effort and monitoring the oxygen consumption with less financial costs [2]. Our results demonstrate that tethered swimming sessions do not induce these negative responses, reinforcing its applicability for conditioning of swimmers. In addition, considering the lack of differences between sessions and the inconclusive correlations with ETL, the present study also demonstrated that the traditional makers of internal load (i.e., [La^-] and RPE] should be used with caution to monitor training periods, mainly when tethered swimming is applied. Considering these observations, coaches can use tethered swimming stimuli during the training routine, without significant biochemical impairments after 24 h.

In conclusion, there is no correlation between ITL-RPE and ITL-[La^-] with ETL. RPE was a sensitive parameter to different intensity domains in an acute training session in tethered swimming, which seems not to be sufficient for changes in [La^-] or biochemical variables, only cortisol was sensitive in distinguish different acute aerobic sessions.

Disclosure of interest

The authors declare that they have no competing interest.

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J.P. Loures[a,*]
C.A.K. Filho[a]
E.Z. Campos[b]
M. Papoti[c]
[a] *Post-Graduation Program in Rehabilitation and functional performance, São Paulo State University, Ribeirão Preto, SP, Brazil*
[b] *Pernambuco State University, Pernambuco, PE, Brazil*
[c] *School of Physical Education and Sports of Ribeirão Preto, São Paulo University, Ribeirão Preto, SP, Brazil*

[*] Corresponding author at: Post-Graduation Program in Rehabilitation and functional performance, São Paulo State University, Av. Bandeirantes, 3900, Monte Alegre, CEP: 14040-907 Ribeirão Preto, SP, Brazil.
E-mail address: jploures@yahoo.com.br (J.P. Loures)

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