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ABSTRACT

The total anaerobic contribution ($AC_{[La-]+PCr}$) is a valid and reliable methodology. However, the active muscle mass plays an important role in the $AC_{[La-]+PCr}$ determination, which might influence its reliability. Thus, this study aimed to investigate the effects of two exhaustive intensities on the reliability of the $AC_{[La-]+PCr}$ during a one-legged knee extension (1L-KE) exercise. Thirteen physically active males were submitted to a graded exercise to determine the peak power output (PPO) in the 1L-KE. Then, two constant-load exercises were conducted to task failure at 100% (TTF_{100}) and 110% (TTF_{110}) of PPO and repeated on a third day. The blood lactate accumulation and the oxygen uptake after exercise were used to estimate the anaerobic lactic and alactic contributions, respectively. Higher values of $AC_{[La-]+PCr}$ were found after the TTF_{100} compared to TTF_{110} ($p = 0.042$). In addition, no significant differences ($p = 0.432$), low systematic error (80.9 mL), and a significant ICC (0.71; $p = 0.004$) were found for $AC_{[La-]+PCr}$ in the TTF_{100}. However, an elevated coefficient of variation was found (13.7 %). In conclusion, we suggest the use of the exhaustive efforts performed at 100% of the PPO with the 1L-KE model but its elevated individual variability must be carefully considered in future studies.

Key-words: High-intensity effort; anaerobic metabolism; MAOD; reliability; performance;

1. INTRODUCTION

High-intensity efforts exacerbate the reliance on non-oxidative energy processes for muscle contraction due to the rapid increase in energy demand (SPENCER; GASTIN, 2001). Therefore, the estimation of the energy supply from phosphocreatine and glucose/glycogen substrates is paramount for athletic monitoring and development during the preparation for short-duration near-maximal events. Numerous field and laboratory-based test protocols have been proposed to estimate the maximal amount of ATP resynthesized anaerobicall1y (i.e., anaerobic capacity) but the acceptance of a gold standard protocol is frequently debated [1].

Traditionally, the anaerobic capacity can be directly determined by the quantification of the anaerobic metabolism by-products in muscle biopsies samples [2] or estimated via indirect calculations [3]. Firstly described by Krogh and Lindhard [4], the oxygen deficit concept attributes the anaerobic energy provision to the difference between the total energy cost for a given task (or later termed theoretical energy demand; TED) and the oxygen consumption ($\dot{V}O_2$) integral area over time (i.e., aerobic supply). Several methods have been suggested to estimate the TED [3, 5] but the most accepted method was proposed by Medbø et al [6]. In the so-called maximum accumulated oxygen deficit (MAOD) protocol, a supramaximal TED is estimated by linear extrapolation of 10-20 submaximal $\dot{V}O_2$ values; however, this protocol is unpractical and has been criticized by others [1, 7]. The concerns regarding the conventional MAOD are mainly related to the application of its procedures, as the number, duration and intensity of the submaximal bouts used to construct the power/speed-$\dot{V}O_2$ relationship have been significantly inconsistent in the literature [1].

An alternative method was proposed by Bertuzzi et al. [8] using a single exhaustive supramaximal effort, which substantially improved the feasibility of the anaerobic capacity estimation and allowed the determination of both anaerobic compartments separately. These authors assumed the anaerobic capacity as the sum of the oxygen equivalents from glycolytic ($E_{[La-]}$) and phosphagen (E_{PCr}) metabolic pathways ($AC_{[La-]+PCr}$), which were attributed to the net blood lactate accumulation and the fast component of the excess post-exercise oxygen consumption ($EPOC_{fast}$), respectively. The $AC_{[La-]+PCr}$ is a valid and reliable methodology both in running [9] and in cycling

[10] across different exercise intensities, with the sensibility to discriminate anaerobic training status [11] and the supplementation with sodium bicarbonate [12]. Additionally, the $AC_{[La-]+PCr}$ is significantly correlated with 30-s Wingate mean (r = 0.79) and peak (r = 0.78) power output [13], swimming performances from 50 to 400 meters (r = 0.68 to 0.91) [14], and performance outcomes derived from the 30-s tethered running (mean force: r = 0.79) [15]. Recently, it was also verified that $AC_{[La-]+PCr}$ remains unchanged under reduced O_2 conditions (i.e., hypoxia), reinforcing its validity as an anaerobic index [16].

Despite the advantages of the $AC_{[La-]+PCr}$, this methodology is influenced by the exercise mode, being higher in running than cycling [17]. Thus, the active muscle mass plays an important role during the estimation of the non-oxidative compartments and must be carefully considered [7]. Indeed, the anaerobic estimations in multi-joint movements could be confounded by adjacent less active muscle groups, altering the capacity for metabolite removal and substrate utilization [18]. In this scenario, the lactate removal from synergetic muscle [19] could underestimate the values of $AC_{[La-]+PCr}$ in whole-body exercises [20]. Therefore, the use of the dynamic one-legged knee extension (1L-KE) model can shed more light on the anaerobic capacity calculations since the $AC_{[La-]+PCr}$ reflects the anaerobic stores from the specific exercised muscles [1, 2, 7, 20]. Anaerobic investigations have already been conducted in the 1L-KE [2, 21] but none of them used the $AC_{[La-]+PCr}$ as a surrogate of the anaerobic capacity.

In this well-controlled exercise mode, the physiological responses (e.g., lactate production, VO_2) reflect the localized metabolic activity of the *quadriceps femoris*, minimizing the non-exercising muscle interference [22, 23]. Thus, the unique physiological characteristics of the 1L-KE must be taken into account in the $AC_{[La-]+PCr}$ calculation as it cannot be extrapolated from other sportive modalities (e.g., running or swimming). Moreover, the $AC_{[La-]+PCr}$ test-retest reliability could also be changed using this single-joint movement pattern and there is an evident need to identify the most robust intensity to its application. The lower locomotor complexity of this task and the specific physiological responses could theoretically improve the reproducibility of the time-to-exhaustion (T_{Lim}) and the associated anaerobic variables; however, this hypothesis remains to be elucidated.

Hence, there is an evident need for better elucidation of the anaerobic indexes using this single-joint design. Therefore, the aim of this study was twofold. Firstly, it was aimed to identify the effects of two different exhaustive intensities on the $AC_{[La-]+PCr}$ calculation using the 1L-KE exercise mode (**Study 1**), considering the premise that there is a range of specific intensities in which $AC_{[La-]+PCr}$ remains valid [9, 17]. In addition, it was also aimed to investigate the test-retest reliability of the $AC_{[La-]+PCr}$ (**Studies2 and 3**) and the associated physiological parameters obtained in these two exhaustive intensities. The study hypothesis is that the $AC_{[La-]+PCr}$ determined in a well-controlled muscle group is a reliable methodology irrespective of the applied exhaustive intensity.

2. MATERIALS AND METHODS

Participants

The sample size was estimated using an online calculator [24] based on published reliability results of the $AC_{[La-]+PCr}$ in cycling [10]. The input parameters were: a statistical power (1-β) of 0.95, a probability α level of 0.05, an expected ICC of 0.96, and two repetitions (k) per subject [25]. The estimation analysis determined that the required number of participants was 11. Thus, thirteen physically active males participated in this study; however, two participants did not attend all evaluations for personal reasons and **Studies1-3** were performed with a different sample. **Table 1** demonstrates the characteristics of the participants included in the different analyses. All participants were free of any health/injury conditions or medications that could limit maximal exercise capacity in the lower limbs. The participants were students of Sports Science related disciplines and attended different sportive modalities at a recreational level and were advised to maintain their usual exercise, sleep, and diet habits throughout the study. Participants were informed of the potential risks of the experimental procedures and provided written informed consent, previously approved by the Human Research Ethics Committee of the School of Physical Education and Sports of Ribeirão Preto, University of São Paulo (60300316.6.0000.5659). All procedures were conducted following guidelines laid out in the Declaration of Helsinki.

insert Table 1 next here

Experimental design

The participants visited the laboratory on three occasions separately by ≥ 24h. In the first visit, participants performed a graded-exercise test (GXT) for 1L-KE peak power

output (PPO) determination. On the second day, two square-waved time-to-task failure efforts (TTF) were performed both at the intensity corresponding to the PPO (TTF_{100}) and at 110% (TTF_{110}). These efforts were conducted on the same leg in a randomized order separated by a 2-hour interval between them. The same pre-determined order of tests was repeated on the third day to assess the test-retest reliability of the variables of interest. Experimental sessions were completed at the same time of day (±1 h) and under standard environmental conditions (temperature 21 ± 1 °C, relative humidity 59 ± 8 %, and barometric pressure 718 ± 1 mmHg). The evaluators provided strong verbal encouragement throughout the exhaustive trials and task failure was considered as the incapability of sustaining the pre-determined kicking frequency of 60 ± 5 RPM for >10s or voluntarily exercise cessation.

Exercise mode: knee-extension ergometer

All experimental sessions were performed with the participant's self-reported dominant leg [26] and their contralateral leg remained attached to the ergometer structure by a non-elastic strap. The dynamic knee extension (DKE) ergometer used is a prototype developed by our research group and previously described [23]. In this exercise model, the participants remain seated in a specific chair with their backs to a conventional mechanically braked cycle ergometer, which has its pedal replaced by a metal bar. This bar is connected to the participants' ankles by a stainless steel boot, and the original movement of cycling is replaced by consecutive knee extensions. Importantly, as the bicycle crankset has a fixed ratchet, the momentum of the flywheel passively returns the leg to the initial position after the active phase of knee extension. Therefore, the *quadriceps femoris* provides the movement pattern with negligible influence of the knee flexors or trunk stabilizers [22, 23]. The exercise intensity adjustment in the DKE ergometer is performed by its pendulum system (i.e. kilopounds) and later converted into Watts (W) after standardized calculations [23]. All participants performed at least one 1L-KE familiarization session was performed in the DKE previous to the data collection. In this session, the positioning of the participants on the ergometer was standardized, ensuring a range of knee extension from 90º to 170º, approximately. In addition, efforts of different intensities and durations were performed (e.g. 1 – 5 min; RPE 5 to 9).

Physiological and metabolic measurements

During all efforts, participants were attached to an oronasal mask (7450 Series Silicone V2, Hans Rudolph Inc., USA). Ventilatory variables were monitored breath-by-breath using a stationary gas analyzer (Quark CPET, Cosmed, Rome, Italy) and later interpolated every 1-s. The equipment was calibrated according to the manufacturer's instructions, using ambient air, a mixture of gases containing 16% O_2 and 5% CO_2 (White Martins, Osasco, SP, Brazil), and a 3 L syringe (Hans Rudolph Inc., USA). During the exhaustive trials, $\dot{V}O_2$ was measured for 5 min at rest (baseline; $\dot{V}O_{2baseline}$) and for 10 minutes after the end of the test to assess the $EPOC_{fast}$. Heart rate (HR) was measured with the Polar H7 sensor (Polar Electro Oy, Kempele, Finland) and the highest values were reported (HR_{peak}). The rate of perceptive exertion (RPE) was determined using a 10-point scale which all participants were familiar with [27]. At baseline ($[La^-]_{baseline}$) and at minutes 3, 5, 7, and 10 after exhaustion, capillary blood (25µl) was sampled from the earlobe using heparinized capillaries tubes and was subsequently frozen at -20 ºC with 50µl of 1% sodium fluoride in Eppendorf type microtubes (1.5 mL). The blood lactate concentration ($[La^-]$) was determined using a YSI 2300 STAT electrochemical lactate analyzer (Yellow Springs Instruments, OH, USA), which has a measurement error of ±2% of the reading or 0.1 mM. The highest values recorded were designed as $[La^-]_{peak}$.

Graded incremental exercise test (GXT)

After a five-minute warm-up at 13 W, a progressive effort with 13 $W \cdot min^{-1}$ increment was applied until the participants' voluntary exhaustion. This protocol was adapted from a previous report [28] and it has demonstrated stable end-exercise $\dot{V}O_2$ values and a high frequency of successful identification of the metabolic thresholds in previous pilot studies conducted in our laboratory. The respiratory quotient (RER) represented the highest values in the ratio between $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$). The mean values of $\dot{V}O_2$ observed during the last 30 seconds of effort were considered the maximum for that effort ($\dot{V}O_{2peak}$). The PPO was attributed to the intensity of the last complete stage or was adjusted according to the equation [29]: $PPO = PC + ([T \cdot D^{-1}] \cdot I)$, where the PC is the power of the last complete stage (W); T is the time the participant remained in the incomplete stage (s); D is the duration of the stages (i.e. 60 s); I is the intensity of the increments (i.e. 13 W).

Constant-load high-intensity time-to-task failure efforts (TTF)

On the second and third days of evaluations, participants were submitted to exhaustive efforts with constant intensity (i.e., 100% [TTF_{100}] or 110% of PPO [TTF_{110}]) until their limit of tolerance (i.e., task failure). These efforts were preceded by the same GXT warm-ups. Effort duration (i.e., time to exhaustion; T_{Lim}) and total work done (TWD) were calculated and attributed as performance parameters. In line with the theoretical concept of the anaerobic capacity [6], these exercise intensities were chosen based on previous reports of our group that demonstrated a ~3-min task failure using, the 1L-KE [21].

In addition, an indirect anaerobic index based on the accumulated oxygen deficit (AOD) was also calculated, assuming the end-exercise $\dot{V}O_2$ values as a proxy for the estimated energy cost and a constant mechanical efficiency [21, 30]. Thus, the total theoretical energy demand for the TTF trials (TED_{100} and TED_{110}) was calculated as the product between the highest 5-s $\dot{V}O_2$ averages and T_{Lim} in each trial [21]. The AOD was assumed as the difference between TED and the $\dot{V}O_2$ integral area ($A\dot{V}O_2$) in the TTF_{100} and TTF_{110} (AOD_{100} and AOD_{110}, respectively).

Energy system contributions

After the TTF_{100} ($AC_{[La-]+PCr}100$) and TTF_{110} ($AC_{[La-]+PCr}110$), the anaerobic contributions were estimated by the $AC_{[La-]+PCr}$ method [8], calculated as the sum of oxygen equivalents from the phosphagen (E_{PCr}) and the glycolytic/lactic metabolism ($E_{[La-]}$). The E_{PCr} was attributed to the $EPOC_{fast}$ area [8, 31], calculated as the product between the first amplitude (A_1) and time constant (τ_1) extracted after a bi-exponential fitting model (Equation 1) of the $\dot{V}O_2$ and the recovery time (10 min) [16] (OriginPro 8.5, Microcal, MA, USA).

$$\text{Equation 1. } \dot{V}O_{2(t)} = \dot{V}O_{2baseline} + A_1[e^{-(t-\delta)\,/\,\tau_1)}] + A_2[e^{-(t-\delta)\,/\,\tau_2)}]$$

Where $\dot{V}O_{2(t)}$ is the rate of oxygen uptake at a time (t); $\dot{V}O_{2baseline}$ is the rate of oxygen uptake at baseline; A is the amplitude; δ is the time delay; and τ is the time constant.

The contribution of the $E_{[La-]}$ was estimated by the net [La^-] accumulation (i.e., $[La^-]_{peak}$ - $[La^-]_{baseline}$), considering the oxygen equivalent of 3 $mL \cdot kg^{-1}$ for each 1.0 mM of lactate accumulated above resting level [8, 31]. In addition, the aerobic contributions (E_{Aer}) were calculated as the subtraction of the $A\dot{V}O_2$ and the $\dot{V}O_{2baseline}$.

Statistical analysis

Descriptive data are presented as mean ± standard deviation. Data dispersion was investigated using box-plot graphs and histograms. The Shapiro-Wilk test was conducted to verify data normality. In **Study 1**, the differences between the physiological and performance parameters obtained in the TTF_{100} and TTF_{110} were performed using the paired *t*-test. The Pearson product-moment correlation coefficient was used to assess the association degree of these variables and was classified as *trivial* < 0.1; *small* 0.1-0.3; *moderate* 0.3-0.5; *large* 0.5-0.7; *very large* 0.7-0.9; and *nearly perfect* > 0.9 [32].

In **Studies2-3**, the test-retest comparison between the dependent variables was also performed using the paired *t*-test. The intraclass correlation coefficient (ICC) and its confidence interval (95% CI) were calculated based on a mixed model of two-way, single measures and absolute agreement (ICC 2.1) [33]. The ICC was interpreted as "*small*" < 0.5; "*moderate*" 0.5-0.75; "*good*" 0.75-0.9; "*excellent*" > 0.9 [33]. Reproducibility was also verified by the typical error (TE) (standard deviation of the differences/√2), coefficient of variation (CV) [34], and by the smallest detectable difference (SDD) [35]. The SDD was calculated as: 1.96 · √2 · SEM. Where SEM (standard error of measurement): SDpooled · √(1-ICC) [36].

In all analyses, effect sizes are reported as Cohen's *d* and calculated as the difference between the test and retest means, divided by the pooled standard deviation. The *d* was classified as "*trivial*" < 0.2; "*small*" 0.2-0.6; "*moderate*" 0.6-1.2; "*large*" 1.2-2.0; "*very large*" 2.0-4.0; "*almost perfect*" > 4.0 [32]. The agreement between the two intensities (**Study 1**) and between the trials (**Studies 2 and 3**) was tested using the graphical analysis of Bland and Altman [37], represented by the means of the residuals (i.e. systematic error; Bias) and its limits of agreement (95% LoA). In all statistical analyses, the significance level was set at $p < 0.05$. The statistical package used was the IBM SPSS Statistics 26 (IBM SPSSInc, Chicago, USA).

3. RESULTS

Study *1: Comparisons between the TTF_{100} and TTF_{110}.*

The variables obtained after the GXT are described in **Table 2**. The physiological, perceptual, and performance obtained after the TTF_{100} and TTF_{110} were described in **Table 3**. Significant differences were found between the two intensities for TED, $\dot{A}VO_2$, and $E_{[La-]}$ ($p < 0.027$) while the other parameters were not different ($p > 0.082$), including the T_{Lim} ($p = 0.119$). The effect sizes ranged from "*trivial*" to "*moderate*" with "*small*" to *very large*" correlations. **Figure 1** show the mean and the individual values of the $AC_{[La-]+PCr}$ in the two TTF efforts. Despite a "*small*" effect size, low mean difference (i.e., Bias) and a "*very large*" correlation, a significant difference was found between the $AC_{[La-]+PCr}100$ and $AC_{[La-]+PCr}110$.

Study *2: Test-retest reliability of the TTF_{100} parameters.*

The results obtained at the moment of exhaustion after the TTF_{100} in the test-retest situation are described in **Table 4**. No significant differences were found between the repeated trials in any of the studied variables ($p > 0.111$). The effect sizes were classified as "*trivial*" or "*small*" for all variables with "*small*" to "*moderate*" ICCs. The CVs were between 6.0 and 37.8%. The $AC_{[La-]+PCr}100$average and individual values are presented separately in **Figure 2** which exhibited a "*small*" effect size and negligible mean differences between the repeated trials and a significant ICC.

Study *3: Test-retest reliability of the TTF_{110} parameters.*

Table 5 depicts the reliability analysis of the TTF_{110} parameters. In agreement with the TTF_{100}, no differences between both repeated conditions were evidenced for the studied variables ($p > 0.086$) with "*trivial*" to "*moderate*" effect sizes. Besides the HR_{peak} and the $\dot{A}VO_2$, the ICCs ranged from "*small*" to "*moderate*". The CVs were between 3.3 and 60.4%. **Figure 3** presents the TFF_{110} values, which exhibited a "trivial" effect size. Importantly, the ICC was not significant in this condition.

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4. DISCUSSION

The present study aimed to investigate the test-retest reliability of the $AC_{[La-]+PCr}$ and its associated physiological variables obtained after two exhaustive efforts (i.e., 100% and 110% of PPO) using a well-controlled exercise model (1L-KE). Contrary to the initial hypothesis, the exercise intensity influenced the $AC_{[La-]+PCr}$ calculation and it was significantly higher after the TTF_{100} (**Study 1**). In the reliability analyses, no significant differences and small systematic errors were found irrespective of the exercise intensity (**Studies 2 and 3**), in line with the study hypothesis. However, the $AC_{[La-]+PCr}100$ exhibited the lowest CV (13.7 *vs* 24.4%) and is the only intensity with a significant ICC. Therefore, the exercise intensity corresponding to 100% of the PPO should be preferred in this exercise mode.

Study *1: Comparisons between the TTF_{100} and TTF_{110}.*

The theoretical concept of the anaerobic capacity suggests that the total amount of ATP formed anaerobically during short-duration exhaustive efforts is independent of the exercise duration in efforts lasting 2-5 minutes [6]. Thus, the exercise intensities chosen in this study were based on previous findings that reported exhaustion in ~2.8 min at 100% of the 1L-KE PPO [21]. Despite elevated mean differences between the TTF_{100} and the TTF_{110}, neither the T_{Lim} (Bias = 31.8 s; p = 0.119) nor the TWD (Bias = 1.2 kJ; p = 0.460) reached statistical significance (**Table 3**), possibly due to an elevated individual variability (**Studies 2-3**). However, contrary to the initial study hypothesis, it was observed a significant impact of the exercise intensity on the $AC_{[La-]+PCr}$ (**Figure 1**), linked to an elevated $E_{[La-]}$ contribuition in the TTF_{100} (**Table 3**).

The present results contrast the previous findings that investigated the influence of exercise intensity on $AC_{[La-]+PCr}$. Zagatto et al. [9] were the first to compare the effects of different running intensities on the determination of the $AC_{[La-]+PCr}$ in physically active men. These authors found that $AC_{[La-]+PCr}$ remains valid (~ 3.5 L) in the range of 100 to 150% of the intensity associated with maximal oxygen uptake ($iV\dot{O}_{2MAX}$) (Zagatto et al. 2016). Similarly, Miyagi et al. (2017) found that exercise intensities at 110-120% of $iV\dot{O}_{2MAX}$ are ideal to determine the $AC_{[La-]+PCr}$ in cycling and found values

close to 4.5 L in mountain-bike cyclists. These conflicting findings could be attributed in part to the higher volume of active muscle mass recruited in these previous studies, which influences neuromuscular and perceived fatigue allowing improved exercise tolerance (i.e., higher T_{Lim}) even at greater intensities [38].

Studies *2 and 3: Test-retest reliability of the TTF_{100} and TTF_{110} parameters.*

The reliability of a physiological test is essential to provide accurate results over time and is especially important in the sports science context, in which the change in the variables of interest are often small [34]. Despite the satisfactory test-retest reproducibility of the average values, an elevated individual variability was found in most of the physiological responses (**Tables 4 and 5**). The heterogeneity of the results can be verified by high CVs and other dispersion parameters (i.e., LoA, SDD, and TE). In addition, the ICCs of the main variables were classified as "*moderate*" or "*small*", limiting their reproducibility. Accordingly, the $AC_{[La-]+PCr}100$ showed a significant moderate ICC (0.71; $p < 0.01$) with a CV of 13.7 % (Figure 2). Despite being more robust than the $AC_{[La-]+PCr}110$ (Figure 3), the high variability of this index must be taken into account in the application of the $AC_{[La-]+PCr}$ in longitudinal studies where a possible training effect is expected.

Conversely, previous findings have demonstrated an adequate index of reliability from $AC_{[La-]+PCr}$. For instance, Zagatto et al. [9] found typical error values comparable to the present study at 115% of the running $iV\dot{O}_{2MAX}$ (270 mL), however, the mean $AC_{[La-]+PCr}$ values were higher (corresponding an estimated CV of ~7 %) than the present study. These authors also observed greater test-retest correlations (ICC = 0.87; $p < 0.001$) and smaller mean differences and LoA (0.90 ± 22 mL). In agreement, Miyagi et al. [10] found even greater reliability of the $AC_{[La-]+PCr}$ in cycling (CV = 4.1 %; ICC = 0.96). A plausible explanation for this disparity may be attributed to the ergometer used in the previous literature in which the participants were fully familiarized (i.e., treadmill and cycle ergometer).

Despite the low complexity of the movement pattern in the DKE ergometer [23], the reliability of the main variables of this study could be impacted due to the unusual nature of the 1L-KE. In addition, TTF efforts are highly dependent on motivation

factors and do not allow pacing strategies which can increase the individual variation in these trials [39, 40]. Indeed, the performance parameters T_{Lim} and TWD showed elevated CV both in the TTF_{100} (25.5 and 26.4 %, respectively) and in the TTF_{110} (38.2 and 38.3 %, respectively) (**Tables 4 and 5**). Although Miyagi et al. [10] reported a satisfactory T_{Lim} CV (4.1 %) in recreationally trained cyclists, Billat et al. [41] verified similar reliability at 100 % of $i\dot{V}O_{2max}$ in sub-elite runners (CV = 25 %). Similarly, Kalva-Filho et al. [42] reported a CV of 18.8% at this same intensity in tethered swimming to exhaustion. Thus, the reliability of the T_{Lim} seems to be dependent on the modality and exercise intensity [40]. In this context, the present findings could be explained, at least to some extent, to the elevated T_{Lim} variability and its influence on the physiological variables studied.

Limitations

A novel aspect of the present study is that the anaerobic contributions were estimated by the $AC_{[La-]+PCr}$during a well-controlled exercise mode. The DKE allows the application of invasive techniques (e.g., muscle biopsy, catheterization) [22] and the neuromuscular assessments of the knee extensors can be performed with a minimal time delay; therefore, the DKE is an interesting tool for different experimental setups. Although, some methodological considerations must be addressed within this study. Firstly, this study was not designed to validate the $AC_{[La-]+PCr}$ against the traditional protocol of the MAOD calculated from submaximal $\dot{V}O_2$ values; therefore, the attainment of maximal anaerobic capacity values could not be inferred here. Moreover, the participants were instructed to maintain their usual nutritional intakes throughout the intervention period. However, the application of supervised dietary records could improve the reliability of the $E_{[La-]}$ associated variables. Furthermore, we only recruited physically active males, and these results could not be extrapolated to athletes or other populations.

In addition, the absence of habituation to the 1L-KE exercise mode could be a possible limitation. To counteract this issue, a familiarization session was performed previous to the experimental data collection. However, the reliability data from this study was lower than other modalities [9, 10]. Therefore, future investigations are advised to perform longer familiarization sessions. Also, the use of testing protocols with known end-point (i.e., fixed distance or time; time trials) could be used in an attempt to enhance the

reliability of the performance indexes [40]. Another important issue that must be pointed out is the use of two exhaustive efforts in the same leg with a 2-h interval. While this timeframe would theoretically be sufficient time for some physiological responses to recover (e.g., VO_2, lactate, and the critical power parameters) [43], it is not sufficient to replenish the muscle glycogen content after high-intensity exhaustive efforts [44]. Thus, we acknowledge that this issue may have impacted the absolute $AC_{[La-]+PCr}$ values and other physiological responses in the second effort. Nevertheless, the intensity of the efforts was randomly chosen and we maintained the same order in the retest condition, ensuring that the second effort was identically influenced for all subjects.

Conclusion

The present findings highlight, for the first time, the influence of exercise intensity on the anaerobic contributions estimated by the $AC_{[La-]+PCr}$ in the knee-extensor exercise. Moreover, the test-retest reliability of the $AC_{[La-]+PCr}$ is limited in exercising involving small muscle mass, mainly in supramaximal intensities (i.e., 110% of PPO). Despite this, no significant differences, low systematic errors, and a significant ICC were found after an exhaustive effort at the maximal GXT intensity (i.e., 100% of PPO) and should be preferred. In this context, although the anaerobic calculations in a well-known muscle group represent an important methodological approach, the investigated parameters must be cautiously extrapolated for future investigations in different experimental settings. We suggest a longer familiarization protocol to reduce the variability of the physiological and performance parameters before applying this methodology to different experimental designs (e.g., training studies and supplementation protocols).

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Figure 1. Mean (A) and individual (B) $AC_{[La-]+PCr}$ values, Bland-Altman plots (C), and correlation analysis (D) after the time-to-task failure at 100% (TTF_{100}) and 110% of PPO (TTF_{110}).

Figure 2 Mean (A) and individual (B) $AC_{[La-]+PCr}$ values, Bland-Altman plots (C), and test-retest correlation analysis (ICC) (D) after the time-to-task failure at 100% (TTF_{100}) in the test and retest condition.

Figure 3. Mean (A) and individual (B) $AC_{[La-]+PCr}$ values, Bland-Altman plots (C), and test-retest correlation analysis (ICC) (D) after the time-to-task failure at 110% (TTF_{110}) in the test and retest condition.

Table 1. Anthropometric characteristics of the participants in the different analyses.

Table 2. Physiological variables obtained after graded exercise test (GXT) in dynamic one-legged knee extension exercise (1L-KE) (n = 13).

Table 3. Physiological and performance parameters obtained after exhaustive efforts performed at 100 and 110% of peak power output (n = 13).

Table 4. Test-retest reproducibility of physiological and performance parameters obtained after exhaustive efforts performed at 100% of peak power output (n = 12).

Table 5. Test-retest reproducibility of physiological and performance parameters obtained after exhaustive efforts performed at 110% of peak power output (n = 11).

Table 1. Anthropometric characteristics of the participants in the different studies.

Study	n	Age (years)	Height (cm)	Body mass (Kg)
1	13	26 ± 3	180.4 ± 8.3	86.3 ± 14.7
2	12	26 ± 3	180.1 ± 8.6	85.7 ± 15.2
3	11	25 ± 2	180.4 ± 7.3	85.4 ± 15.7

Table 2. Physiological variables obtained after graded exercise test (GXT) in one-legged knee-extensor exercise (1L-KE) (n = 13).

Variable	**Mean**	**SD**
Duration (s)	295	48
PPO (W)	64	11
VO_{2peak} ($mL \cdot min^{-1}$)	1025	310
RER (a.u.)	1.22	0.15
HR_{peak} ($beats \cdot min^{-1}$)	140	14
RPE (a.u)	9	1
$[La^-]_{peak}$ (mM)	5.64	1.56

Note. PPO = peak power output obtained in the graded exercise test; VO_{2peak} = highest 30 s VO_2 averages; VE = minute ventilation; RER = respiratory exchange ratio; RPE = rate of perceived exhaustion; HR_{peak} = highest 30 s averages; $[La^-]_{peak}$ peak blood lactate concentration.

Table 3. Physiological and performance parameters obtained after exhaustive efforts performed at 100 and 110% of peak power output (n = 13).

	TTF_{100}	TTF_{110}	*p*-value	*d*	*R*	Bias (95% LoA)
T_{Lim} (s)	174.2 ± 52.7	148.0 ± 55.2	0.119	0.58	0.46	31.8 (-76.1 to 139.6)
TWD (kJ)	11.09 ± 3.56	10.31 ± 3.80	0.460	0.33	0.50	1.20 (-5.75 to 8.14)
RPE (a.u.)	8.54 ± 1.51	8.69 ± 1.03	0.613	-0.16	0.71**	-0.33 (-2.07 to 1.41)
HRpeak (bpm)	137.6 ± 14.5	132.9 ± 11.3	0.163	0.44	0.65*	5.82 (-15.55 to 27.19)
[La^-]peak (mM)	6.08 ± 0.96	5.33 ± 1.18	0.057	0.76	0.29	0.83 (-1.72 to 3.39)
$\dot{V}O_{2peak}$ (mL·min^{-1})	1629.6 ± 377.2	1560.7 ± 363.6	0.342	0.14	0.77**	51.1 (-446.5 to 548.6)
TED (mL)	4778.0 ± 1955.1	3775.3 ± 1439.4	**0.025**	0.60	0.69**	1076.2 (-1772.6 to 3925.0)
E_{Aer} (mL)	1930.0 ± 698.0	1647.9 ± 602.9	**0.027**	0.45	0.82**	300.2 (-516.5 to 1116.9)
AOD (mL)	2848.0 ± 1418.8	2127.4 ± 1137.2	0.082	0.58	0.44	776.0 (-1995.7 to 3547.6)
E_{PCr} (mL)	488.4 ± 198.1	487.5 ± 231.9	0.989	-0.04	0.37	-8.21 (-501.4 to 484.7)
$E_{[La]}$ (mL)	1322.8 ± 360.7	1151.8 ± 368.5	**0.023**	0.42	0.79**	159.2 (-317.3 to 635.7)

T_{Lim}: time-to-exhaustion. TWD: total work done (kJ). RPE: rate of perceived exertion. HRpeak: peak heart rate value. $\dot{V}O_{2pico}$ (mL·min^{-1}): peak 5-s $\dot{V}O_2$ averages. E_{Aer} (mL): $\dot{V}O_2$ *vs* T_{Lim} integral area subtracting the $\dot{V}O_{2baseline}$. TED (mL): theoretical energetic demand ($\dot{V}O_{2peak}$ *vs* T_{Lim}. AOD (mL): accumulated oxygen deficit (TED - E_{Aer}). [La^-] peak: peak blood lactate concentration. E_{PCr}: anaerobic alactic equivalent. $E_{[La]}$: anaerobic lactic equivalent. P-values reported from paired *t*-tests and bold text indicate statistically significant results. *d*: Cohen's *d* effect size. *R*: Pearson correlation. Bland-Altman analysis with residuals and 95% level of agreement.

Table 4. Test-retest reproducibility of physiological and performance parameters obtained after exhaustive efforts performed at 100% of peak power output (n = 12).

	Test	Retest	*p*-value	*d*	Bias (95% LoA)	ICC (95% CI)	SDD	SEM	CV%
T_{Lim} (s)	174.3 ± 55.1	176.3 ± 49.8	0.915	-0.04	-2.0 (-125.7 to 121.7)	0.30 (-0.37 to 0.74)	122.2	44.1	25.5
TWD (kJ)	7.13 ± 2.09	7.95 ± 4.09	0.787	-0.08	-0.32 (-8.52 to 7.87)	0.49 (-0.12 to 0.82)	8.04	2.90	26.4
RPE (a.u.)	8.42 ± 1.50	8.92 ± 1.00	0.111	-0.39	-0.50 (-2.46 to 1.46)	0.66 (0.20 to 0.89)**	2.07	0.75	8.2
HRpeak (bpm)	137.8 ± 15.2	133.7 ± 11.1	0.247	0.31	4.09 (-18.60 to 8.19)	0.61 (0.12 to 0.87)*	23.0	8.3	6.0
[La^-]peak (mM)	6.16 ± 0.96	5.72 ± 1.17	0.241	0.40	0.43 (-1.98 to 2.85)	0.32 (-0.24 to 0.74)	2.47	0.89	14.7
$\dot{V}O_{2peak}$ (mL·min-1)	1654.7 ± 382.5	1727.4 ± 365.6	0.464	-0.19	-72.72 (-723.99 to 234.96)	0.61 (0.87 to 0.98)*	644.4	232.5	13.9
TED (mL)	4856.8 ± 2020.4	5222.9 ± 2483.8	0.572	-0.16	-366.1 (-4630.2 to 3898.0)	0.55 (-0.01 to 0.85)*	4200.3	1515.3	30.5
E_{Aer} (mL)	1980.9 ± 703.5	2242.2 ± 1418.5	0.439	-0.23	-261.3 (-2472.5 to 1950.0)	0.50 (-0.07 to 0.83)*	2194.4	791.7	37.8
AOD (mL)	2875.9 ± 1478.2	2980.8 ± 1249.4	0.810	-0.08	-104.82 (-2998.1 to 2788.5)	0.44 (-0.19 to 0.80)	2843.9	1026.0	35.6
E_{PCr} (mL)	480.0 ± 204.5	532.7 ± 265.1	0.356	-0.22	-52.7 (-424.4 to 134.1)	0.68 (0.22 to 0.90)**	371.2	133.9	26.5
$E_{[La]}$ (mL)	1329.8 ± 375.8	1196.2 ± 271.0	0.242	0.41	133.6 (-600.4 to 867.6)	0.34 (-0.22 to 0.74)	739.5	266.8	21.0

T_{Lim}: time-to-exhaustion. TWD: total work done (kJ). RPE: rate of perceived exertion. HRpeak: peak heart rate value. $\dot{V}O_{2pico}$ ($mL \cdot min^{-1}$): peak 5-s $\dot{V}O_2$ averages. E_{Aer} (mL): $\dot{V}O_2$ *vs* T_{Lim} integral area subtracting the $\dot{V}O_{2baseline}$. TED (mL): theoretical energetic demand ($\dot{V}O_{2peak}$ *vs* T_{Lim}. AOD (mL): accumulated oxygen deficit (TED - E_{Aer}). [La^-]peak: peak blood lactate concentration. WAla: anaerobic alactic equivalent. WLa: anaerobic lactic equivalent. ICC: intraclass correlation coefficient ([2.1] = 2-way mixed. absolute agreement. single values); Bias (95% LoA): Bland-Altman analysis with residuals and 95% level of agreement. SDD: smallest detectable difference. TE: typical error. CV: coefficient of variation. *: $p < 0.05$. **: $p < 0.01$.

Table 5. Test-retest reproducibility of physiological and performance parameters obtained after exhaustive efforts performed at 110% of peak power output (n = 11).

	Test	Retest	*p*-value	*d*	Bias (95% LoA)	ICC (95% CI)	SDD	SEM	CV%
T_{Lim} (s)	152.5 ± 59.3	150.5 ± 39.5	0.940	0.04	1.91 (-158.6 to 162.4)	0.366 (-0.332 to 0.901)	163.1	58.8	38.2
TWD (kJ)	10.73 ± 3.67	11.12 ± 4.46	0.831	-0.09	-0.39 (-12.0 to 11.2)	0.059 (-0.562 to 0.710)	11.65	4.20	38.3
RPE (u.a.)	8.64 ± 1.03	9.18 ± 0.87	0.052	-0.57	-0.55 (-2.15 to 1.06)	0.558 (0.021 to 0.854)*	1.76	0.63	6.5
HRpeak (bpm)	133.8 ± 11.9	138.1 ± 12.6	0.050	-0.35	-4.29 (-16.84 to 8.25)	0.823 (0.410 to 0.951)**	14.30	5.16	3.3
[La^-]peak (mM)	5.28 ± 1.28	5.60 ± 1.30	0.359	-0.25	-0.31 (-2.46 to 1.83)	0.638 (0.121 to 0.887)*	2.14	0.77	14.2
$\dot{V}O_{2peak}$ (mL·min-1)	1518.3 ± 303.5	1617.4 ± 277.3	0.209	-0.34	-99.1 (-578.2 to 380.1)	0.630 (0.127 to 0.882)*	490.1	176.8	11.0
TED (mL)	3783.4 ± 1482.4	4167.6 ± 1665.9	0.544	-0.24	-384.1 (-4355.8 to 3587.6)	0.183 (-0.480 to 0.693)	3950.7	1425.3	36.0
E_{Aer} (mL)	1651.9 ± 646.3	1764.5 ± 838.4	0.345	-0.15	-112.6 (-851.4 to 626.2)	0.873 (0.615 to 0.964)**	739.4	266.8	15.6
AOD (mL)	2131.6 ± 1179.3	2403.1 ± 1063.5	0.652	-0.24	-271.5 (-4068.7 to 3525.6)	0.546 (-0.138 to 0.96)	3870.4	1396.3	60.4
E_{PCr} (mL)	442.8 ± 163.4	571.6 ± 169.5	0.086	-0.77	-128.9 (-569.1 to 311.4)	0.074 (-0.367 to 0.579)	444.1	160.2	31.3
$E_{[La]}$ (mL)	1132.1 ± 400.2	1086.6 ± 426.8±	0.675	0.11	45.5 (-646.8 to 737.9)	0.653 (0.109 to 0.894)*	675.5	243.7	22.5

T_{Lim}: time-to-exhaustion. TWD: total work done (kJ). RPE: rate of perceived exertion. HRpeak: peak heart rate value. $\dot{V}O_{2pico}$ (mL·min^{-1}): peak 5-s $\dot{V}O_2$ averages. E_{Aer} (mL): $\dot{V}O_2$ *vs* T_{Lim} integral area subtracting the $\dot{V}O_{2baseline}$. TED (mL): theoretical energetic demand ($\dot{V}O_{2peak}$ *vs* T_{Lim}. AOD (mL): accumulated oxygen deficit (TED - E_{Aer}). [La^-]peak: peak blood lactate concentration. WAla: anaerobic alactic equivalent. WLa: anaerobic lactic equivalent. ICC: intraclass correlation coefficient ([2.1] = 2-way mixed. absolute agreement. single values); Bias (95% LoA): Bland-Altman analysis with residuals and 95% level of agreement. SDD: smallest detectable difference. TE: typical error. CV: coefficient of variation. *: $p < 0.05$. **: $p < 0.01$.

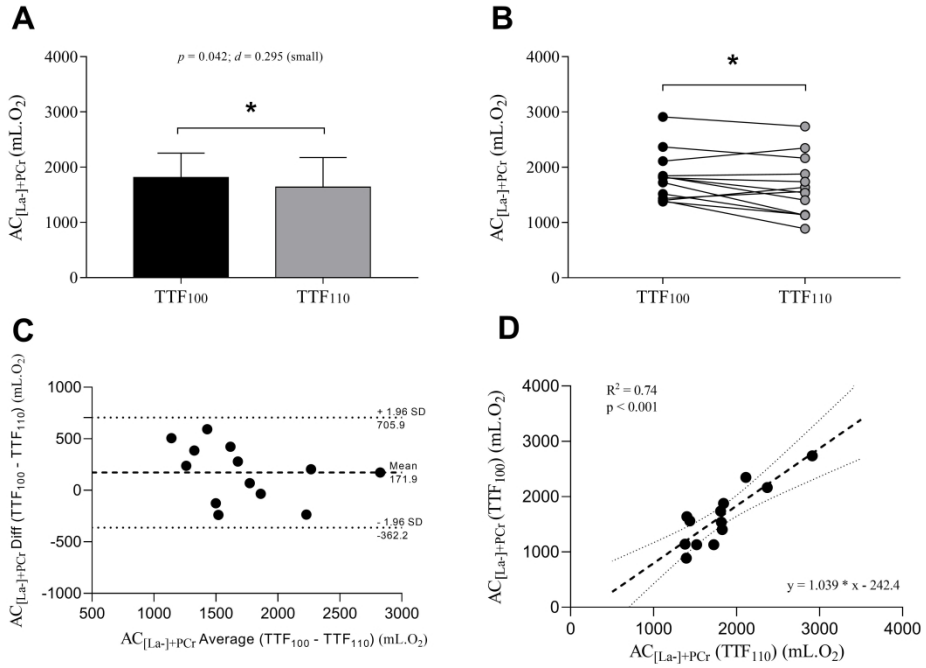

Figure 1. Mean (A) and individual (B) AC[La-]+PCr values, Bland-Altman plots (C), and correlation analysis (D) after the time-to-task failure at 100% (TTF100) and 110% of PPO (TTF110)

220x157mm (600 x 600 DPI)

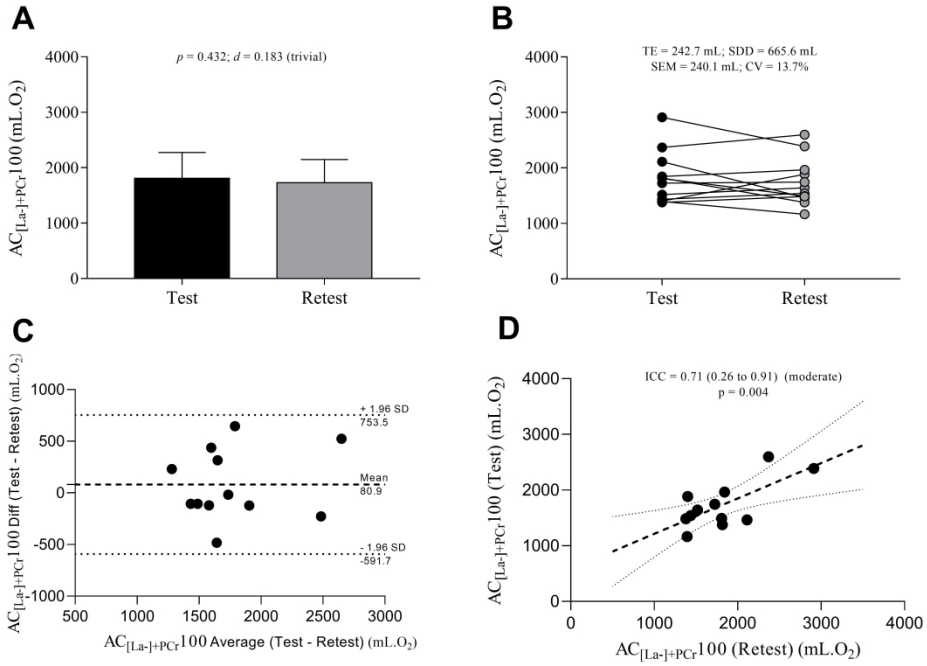

Figure 2. Mean (A) and individual (B) AC[La-]+PCr100 values, Bland-Altman plots (C), and test-retest correlation analysis (ICC) (D) after the time-to-task failure at 100% (TTF100) in the test and retest condition

220x156mm (600 x 600 DPI)

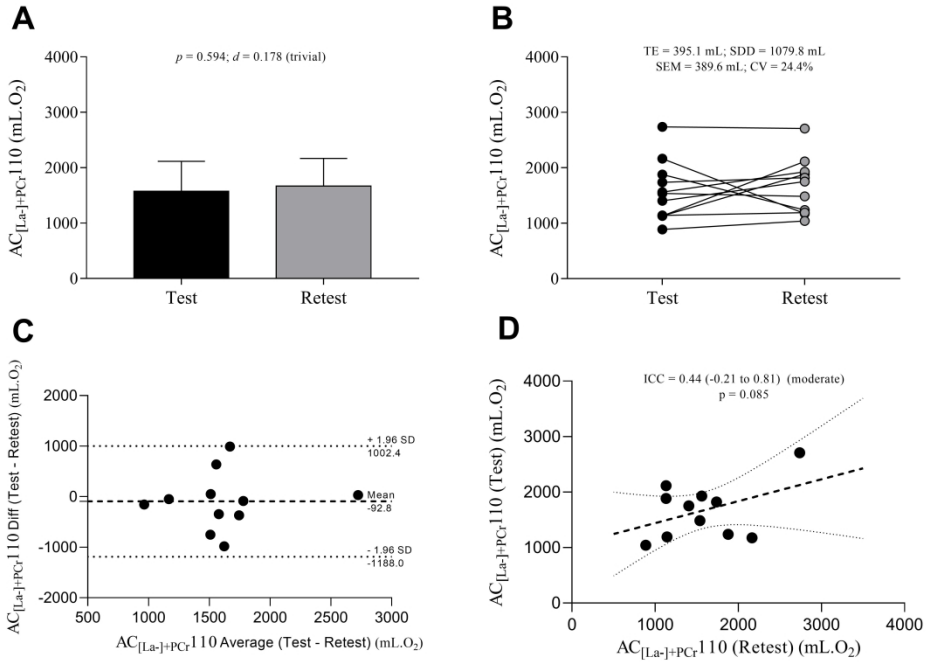

Figure 3. Mean (A) and individual (B) AC[La-]+PCr110 values, Bland-Altman plots (C), and test-retest correlation analysis (ICC) (D) after the time-to-task failure at 110% (TTF110) in the test and retest condition

220x156mm (600 x 600 DPI)