Comparison between the use of saliva and blood for the minimum lactate determination in arm ergometer and cycle ergometer in table tennis players

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ABSTRACT

The aim of this study was to verify if it is possible to determine the lactate minimum test (LMT) by saliva sodium (Na⁺), potassium (K⁺) and lactate (LAC) concentrations in arm ergometer and cycle ergometers. Eight male international-level table tennis players participated in this study. To induce increases of lactate concentration in both ergometers, 30 seconds maximal tests were used with maximal force application in constant 102 rpm in isokinetic arm ergometer (Cybex UBE 2432), and loads of 7.5% of body weight (Kp) in cycle ergometer (mechanical Monark). After the anaerobic stimulus in arm ergometer, the incremental test was applied at constant 60 rpm, started at 49 watts and increment loads of 16 watts each three minutes. The LMT intensity was determined with the analysis of the blood lactate (LACmin_arm) and the saliva concentrations of sodium (Na⁺min_arm-saliva) and potassium (K⁺min_arm-saliva). For the cycle ergometer, the incremental test started with an intensity of 85 watts and increments of 17 watts at constant speed of 70 rpm. The stages were also of three minutes. The LACmin was determined using blood and saliva samples (LACmin_cycle, Na⁺min_cycle-saliva, K⁺min_cycle-saliva, and LACmin_cycle-saliva, respectively). In both ergometers, the intensity obtained in lactate minimum test was correspondent to zero derived polynomial adjustments by metabolite concentrations versus exercise intensities. The statistical analysis included one way ANOVA test, paired t-test and Pearson’s correlations. For all tests applications, the significance level was prefixed at 5%. The several LACmin determinations using blood and saliva samples did not show significant differences in arm and cycle ergometers (LACmin_arm 91.71 ± 12.43; Na⁺min_arm-saliva 71.99 ± 23.42; K⁺min_arm-saliva 79.67 ± 17.72; LACmin_cycle 157.68 ± 13.48; LACmin_cycle-saliva 135.49 ± 33.21 watts). However, these intensities presented no significant correlations. These results showed that determination of the LMT by saliva lactate, sodium and potassium concentrations seems not to be possible with the use of isokinetic arm ergometer and cycle ergometers.

INTRODUCTION

The blood lactate concentration [LAC] has shown to be excellent tool for the training monitoring[9,11], prediction of the endurance performance[12,13] and training prescription[14,15]. With the analysis of the blood lactate concentration, it is possible to determine the anaerobic threshold (AnT), which represents an aerobic evaluation parameter[16,17]. The anaerobic threshold corresponds to the exercise maximal intensity in which equilibrium between production and removal of blood lactate in long-duration activities is verified[6,9,11]. Heck et al.[9] evaluated the blood lactate concentration along the running exercise session with constant load and observed that, regardless the individual aerobic capacity, the maximal lactate steady state (MLSS) was equivalent to 4.0 mM. The authors reported that the lactate production/removal ratio in humans finds its dynamic equilibrium in maximal concentrations of 4.0 mM, with range of 3.0 to 5.5 mM[18]. However, in the study of Heck et al.[18] only six out of the 16 participants presented lactate concentrations close to 4.0 mM (3.81; 4.00; 4.01; 3.74; 3.89; 4.00 mM), once it deals about average value rather than individual determination, thus enabling errors in the performance prediction and/or training prescription. Beneke and Von Duvillard[19] showed that the blood lactate maximum equilibrium point depends on the sportive modality performed, where different stabilization values of this metabolite are found in different sportive modalities. Beneke and Von Duvillard[19] also reported that the concentration in which the blood lactate stabilization occurs depends on the amount of muscular mass involved on the performance of the movement motor standard, thus corroborating for the use of protocols to measure AnT with individual concentrations rather than constant values.

Tegtbur et al.[13] adapted findings of Daves and Gass[14], who reported the possibility of estimating AnT through the intensity corresponding to the lowest lactate concentration obtained in incremental test after hyperlactacidemia induction (figure 1). Tegtbur et al.[13] adapted this protocol for runners and achieved estimating AnT using this procedure. Macintosh et al.[15] corroborated the result obtained by Tegtbur et al.[13] by presenting the lactate minimum test as validated and reproducible, thus enabling estimating the MLSS intensity through this protocol in cycle ergometer. Simões et al.[16] corroborate these findings by reporting the possibility of obtaining the aerobic capacity using this protocol, also showing to be possible to determine this parameter with the use of the glycemia. However, the determination of AnT and protocols with similar physiological phenomena that use the lactate concentration for their measurement are specific invasive procedures for the collect of biological material, generally blood.

Some researchers have proposed the determination of AnT through metabolites present in the saliva[17,18] and lactate present in the sweat[19]. Chicarro et al.[17] showed that the electrolytes in saliva such as chloride (Cl⁻), sodium (Na⁺) and potassium (K⁺) might be used for the determination of the anaerobic threshold in incremental protocols. The same group of researchers[18] later confirmed these findings. Segura et al.[20] reported the possibility of determining AnT through the saliva lactate concentration in incremental protocol using cycle ergometer. These authors found good correlation between AnT obtained with blood and AnT obtained with saliva (r = 0.81). Ben-Aryeh et al.[22] analyzed the lactate response in saliva in incremental exercise in Wingate test. Increases on the saliva lac-
tate concentration were verified, similarly to the blood lactate response. Pérez et al.'(16) reported the possibility of determining the maximal lactate steady state through saliva. The authors performed the study in cycle ergometer and verified high correlations between MLSS in blood and saliva when expressed in relation to VO₂ (r = 0.89) and power (0.92). The maximum variation to be considered for the MLSS determination in blood is of 1.0 mM. However, the researchers reported that to determine MLSS using saliva, the variation of lactate to be used should not exceed 0.8 mM. Mendes et al.'(23) determined the anaerobic threshold through incremental protocol. AnT was obtained through visual examination of the lactate concentration behavior in saliva versus exercise intensity in cycle ergometer. The authors described that the saliva lactate may be used to determine AnT in incremental protocol in cycle ergometer. Thus, the objective of this work is to verify the use of sodium (Na⁺ saliva), potassium (K⁺ saliva) and lactate (LAC saliva) present in saliva in substitution to the blood lactate for the identification of AnT using the minimum lactate protocol in cycle ergometer and arm ergometer.

MATERIAL AND METHODS

Participants

Eight male international-level table tennis players who play for the ADM team – Marilia, SP, participated in this study. The participants presented as characteristics (average ± standard deviation): age of 18.13 ± 2.47 years; height of 176 ± 10 cm; body weight of 67.03 ± 10.67 kg; body fat of 14.70 ± 7.13% and body mass index (BMI) of 21.70 ± 2.90 kg/m². The methodological procedures were recorded with the aid of a JVC DV-9800 digital camera. The digital camera pictures acquisition frequency was of 60 Hz in which were later analyzed for the determination of the Wingate test variables (maximal load, intermediate load and fatigue index) determined each two seconds. A progressive test in cycle ergometer (Monark, Brazil) started eight minutes after the Wingate test with initial intensity of 85 watts and increment of 17 watts each stage of three minutes. The rotation was kept as constant at 70 rpm during the entire test. The test was interrupted with voluntary exhaustion or the non-maintenance of the rotation of 70 rpm. Samples of blood and saliva were collected after each exercise stage with no exercise interruption. The LACmin intensities were determined with blood samples (LACmin blood) and samples of sodium (Na⁺min saliva) and potassium (K⁺min saliva) present in saliva.

Minimum lactate, minimum sodium and minimum potassium tests in arm ergometer

A four-minutes duration warm up exercise with intensity of approximately 85 watts and constant rotation of 70 rpm was performed before the test. The Wingate test was applied five minutes after in cycle ergometer for the hyperlactacidemia induction. The test consisted of performing the exercise in maximum load for a period of 30 seconds with overload of 7.5% of the body weight. The Wingate test in cycle ergometer started with no overload, which was added shortly after its beginning. The recording of the exercise time only started after preestablished load had been reached. During the entire test, the participants were verbally encouraged to perform maximal exercise. After performance of 30-seconds effort, samples of blood and saliva were collected at one, three, five and seven minutes. The revolutions obtained in the test were recorded with the aid of a JVC DV-9800 digital camera. The digital camera pictures acquisition frequency was of 60 Hz in which were later analyzed for the determination of the Wingate test variables (maximal load, intermediate load and fatigue index) determined each two seconds. A progressive test in cycle ergometer (Monark, Brazil) started eight minutes after the Wingate test with initial intensity of 85 watts and increment of 17 watts each stage of three minutes. The rotation was kept as constant at 70 rpm during the entire test. The test was interrupted with voluntary exhaustion or the non-maintenance of the rotation of 70 rpm. Samples of blood and saliva were collected after each exercise stage with no exercise interruption. The LACmin intensities were determined with blood samples (LACmin blood) and samples of sodium (Na⁺min saliva) and potassium (K⁺min saliva).

Minimum lactate test in cycle ergometer after hyperlactacidemia induction (LACmin cycle)

For both tests applied, the minimum lactate intensities with blood samples (LACblood) and minimum lactate in saliva (LACsaliva), minimum sodium (Na⁺min saliva) and minimum potassium (K⁺min saliva) were correspondent to zero derived polynomial adjustments by metabolite concentrations versus exercise potence (P), plotted through the computational program Origin 4.0 (Microcal™) (figure 1).

**Fig. 1** – Minimum lactate intensity determination through minimum lactate test in cycle ergometer after hyperlactacidemia induction (LACmin cycle) corresponding to participant 2.
**Blood analysis**

The blood samples (25 µl) were collected from the earlobe of participant with capillaries calibrated and transferred into **Eppendorf** tubes of 1.5 ml containing 50 µl of NaF (sodium fluoride – 1%). The homogenized (25 µl) was injected in lactimeter YSI model 1500 Sport (Ohio, USA) for lactacidemia analysis. The blood lactate results are expressed in mM.

**Collect and analysis of saliva samples**

For the collecting of saliva samples, mint chewing gum (Trident, Adams) was administered 10 seconds before the end of each stage in order to stimulate saliva secretion. The chewing gum was collected after saliva collecting. The saliva was collected in disposable plastic cup and transferred into **Eppendorf** tube of 1.5 ml.

For the measurement of the saliva lactate, 25 µl of saliva was injected in electrochemical lactimeter YSI, model 1500 Sports (Ohio, USA). The results are expressed in mM. The saliva samples collected for the lactate determination were analyzed shortly after collecting.

The determination of the sodium (Na⁺) and potassium (K⁺) concentrations in the saliva were performed by means of the dilution of 50 µl of saliva in 2.5 ml of distilled water and the homogenized was later analyzed in flame photometer **Pegasus II**. The results are expressed in mEq/L. The analyses of the saliva samples for the determination of the sodium and potassium concentrations were performed at the same day as the execution of the test.

**Statistical analysis**

The analysis of variance (ANOVA – one way) was used for comparisons between LMT intensities determined with blood and saliva samples in their respective ergometers, followed by post hoc Newman-Keuls test, whenever necessary. For the analysis of the lactate concentrations obtained with blood and saliva samples, the paired t-Student test was used. The Pearson correlation test was applied between all variables obtained in each ergometer. For the analysis of the results, the statistical program Statistica for Windows 5.1 (Statsoft, Inc. 1995) was used. In all cases, the significance level was prefixed at p < 0.05. The results were expressed as average ± standard deviation.

**RESULTS**

Table 1 presents the values of the maximum load, intermediate load, maximum load corrected by the body weight, intermediate load corrected by the body weight and fatigue index after Wingate tests. The values presented in table 1 correspond to results obtained in the Wingate test in cycle ergometer and in the Wingate test in arm ergometer.

<table>
<thead>
<tr>
<th>Wingate in arm ergometer</th>
<th>Wingate in cycle ergometer</th>
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<tbody>
<tr>
<td>Pmax (watts)</td>
<td>772.17 ± 94.07</td>
</tr>
<tr>
<td>Pinter (watts)</td>
<td>602.70 ± 72.33</td>
</tr>
<tr>
<td>Pmax/kg (watts/kg)</td>
<td>11.60 ± 0.76</td>
</tr>
<tr>
<td>Pinter/kg (watts/kg)</td>
<td>9.06 ± 0.76</td>
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<tr>
<td>IF (%)</td>
<td>42.69 ± 5.87</td>
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Table 2 presents the highest values found for blood peak lactate (LAC peak-b) blood, lactate in saliva (LAC peak-saliva), sodium (Na peak-saliva) and potassium (K peak-saliva) after Wingate tests in the ergometers used. The lactate concentration determined in the arm ergometer showed to be significantly lower than lactate determined in cycle ergometer (p = 0.001), both serum measurements. However, the variables analyzed with saliva presented no differences between ergometers.

<table>
<thead>
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<th>TABLE 2</th>
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<tr>
<td>Results found for blood peak lactate (LAC peak-b) and saliva peak lactate (LAC peak-saliva), peak sodium (Na peak-saliva) and peak potassium (K peak-saliva) in saliva, collected in the Wingate test performed in arm ergometer and cycle ergometer</td>
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<tr>
<td>LAC peak-b (mM)</td>
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<tr>
<td>Arm ergometer</td>
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<td>Cycle ergometer</td>
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* p < 0.01 in relation to arm ergometer.

**Fig. 2** – Curves corresponding to the metabolite concentrations and exercise intensities in arm ergometer for the exercise intensity determination through minimum lactate protocol. **Figure a** corresponds to the potassium concentration (K peak-saliva), **figure b** the sodium concentration (Na peak-saliva) and **figure c** the blood lactate concentration (LAC blood) corresponding to participant 2. + Metabolite behavior; — second order polynomial adjustment.
Figures 2 and 3 present the behavior of the potassium, sodium and lactate concentrations in saliva and blood lactate measured at the incremental stage of the lactate minimum test in arm ergometer and cycle ergometer, respectively, for participant 1.

Table 3 presents the exercise intensities obtained through minimum lactate protocol for lactate, sodium and potassium samples, the concentrations of these metabolites in the LACmin intensities and the polynomial regression coefficients for blood and saliva samples in both ergometers. No significant differences were found between exercise intensities corresponding to LMT with the metabolites used. However, no significant correlation between these variables was found both for samples in arm ergometer and for samples in cycle ergometer (table 4).

### DISCUSSION

The use of the blood lactate has shown to be a good tool in the evaluation, prescription and monitoring of the sportive training, especially for high-level athletes who need accuracy and sensitivity in the determination of these parameters. Pyne et al. used the blood lactate concentration for the training monitoring of world-ranked swimmers. The authors determined the anaerobic threshold through seven maximal swimming of 200 m in four different occasions of the training planning for a period of eight months. It was demonstrated that significant changes on the lactate tolerance occur in the 200 m performance and on the anaerobic threshold during the analyses performed, showing that the use of the blood lactate is a tool sensible to training adaptations also in high-level athletes.

Billat reports in a reviewing work that the blood lactate concentration may be used for the training prescription of long-duration runnings, once training at intensities corresponding to the range of 2 to 3 mM would represent the ideal intensity for the marathon. In lower volume exercises such as 10 to 16 km, the ideal intensity for the aerobic fitness training and performance seems to occur at intensity corresponding to 4 mM. The blood lactate concentration...
may also be used for the evaluation of the anaerobic fitness in short-duration supra-maximal exercise and with the increase on the energetic demand per time unit in these activities, there is a higher demand of the ATP-CP and glycolytic systems for the re-synthesis of adenosine triphosphate (ATP), also occurring a higher lactate production and release into the blood stream[46]. The lactate minimum test initially proposed by Davis and Gass[14] has been used to predict the intensity corresponding to the anaerobic threshold as well as the intensity of maximal lactate steady state, once they are similar metabolic phenomena but distinct physiological phenomena[13,15]. The lactate minimum test has not been well accepted by some laboratories[24,25], although recent studies have presented the lactate minimum test as valid and reproducible, being used to measure the aerobic fitness and to estimate the MLSS intensity[15,26-29]. This protocol analyzes the behavior of the lactate concentration in an incremental test with previous anaerobic stimulus for the hyperlactacidemia induction, considering the minimum lactate concentration found in the incremental phase as the LACmin intensity[17,18]. Simoses et al.[16,26] reported the possibility of determining the LACmin intensity by analyzing the glycemia behavior (GLUCOSEmin) instead of the blood lactate.

The determination of the intensity corresponding to the anaerobic threshold through the blood lactate concentration has shown to be reproducible, reliable and sensible to adaptations resulting from physical training. However, a small sample of blood is required for the determination of this parameter, thus being characterized as an invasive procedure. The number of researches using non-invasive procedures has increased significantly in the last years aiming at facilitating the estimation of the anaerobic threshold. Some authors have investigated the use of different metabolites, body compartments and alternative electrolytes such as blood glucose samples to estimate the anaerobic threshold intensity[16,26-29]. In our work, we used sodium, potassium and lactate concentrations measured in the saliva as possible electrolytes and metabolites to replace the blood lactate in the determination of the minimum lactate intensity. The saliva electrolytes responses during exercise have already been previously investigated by other researchers. Salminen and Konttinen[31] reported an increase on the sodium and potassium concentrations in saliva after exercise and the fact that increases on these electrolytes as well as lactate in saliva occurred in sub-maximal incremental exercise was later corroborated[22]. From these findings, other studies emphasized the possibility of estimating the anaerobic threshold and the maximal blood lactate steady state using saliva samples[17-19,23]. In the present work, the intensities corresponding to LACmin cycle, LACmin arm, Na+min cycle-saliva and K+min cycle-saliva; and LACmin arm, LACmin arm-saliva, Na+min arm-saliva and K+min arm-saliva presented no significant differences in the ergometers used. However, no significant correlation was found between intensities determined with saliva samples and LACmin determined with blood samples, also presenting low regression coefficients, except for K+min arm (0.92 ± 0.05). The participants of this study were well-trained table tennis athletes. How-ever, the athletes’ sportive characteristic seems not to influence the results obtained, once the comparison between results were performed specifically for each ergometer, performing the same exercise. Table tennis is characterized by powerful movements of the lower limbs associated to quick strokes of the upper ones[32], reason why the ergometers were selected. Chicarro et al.[17] determined the anaerobic threshold using sodium, potassium and lactate concentrations in saliva in incremental protocol in cycle ergometer. The authors found no significant differences between AnT determined with saliva and blood samples and high correlations of AnT determined with saliva samples and AnT determined through blood lactate (r = 0.82) and the catecholamines threshold (r = 0.75). Mendes et al.[22] determined AnT with blood and saliva samples and through the ventilatory method in incremental protocol in cycle ergometer with analysis and saliva samples collecting procedures (Na+, K+ and lactate) similar to procedures used in this work. No statistical differences were verified between AnT measured with different samples and methods, presenting significant correlation between saliva samples with AnT measured through lactate and through ventilatory method.

The salivary secretion is influenced by hormonal stimuli in rest and during exercise[17,29]. The action of parasympathetic hormones stimulates the saliva secretion resulting in a hypococoncentrated compound with low concentrations of organic substances[34], while the sympathetic stimulation induces to saliva secretion with higher concentration of organic substances, making the medium to become hyperconcentrated[33,34]. In physical exercises, an increase on the secretion of sympathetic hormones occurs, especially catecholamines[39] that cause an increase on the sodium, potassium and lactate concentrations in saliva[17,18,22]. In exercises with progressive loads, the increase on the concentration of these electrolytes and lactate is verified in saliva proportionally to the effort intensity, thus enabling the determination of AnT and MLSS through these substances. However, the process to remove these substances from saliva and the salivary gland response time after hormonal stimuli, predominant factors in the determination of the exercise intensity through the lactate minimum test, have still been not much investigated. The metabolites investigated in saliva did not present the same behavior as the blood lactate, making the utilization of these substances in the minimum lactate protocol difficult. The results obtained lead us to speculate that a disconnection in the electrolytes and lactame removal process in saliva or on the salivary gland response seems to occur after sympathetic stimulus posterior to the anaerobic stimulus for the induction of the increase on these concentrations, as in the case of the lactate minimum test. This possible alteration, which we believe to be caused by the Wingate test, in other words, by the anaerobic stimulus required when the protocol used is the lactate minimum test, hinders the use of sodium, potassium and lactate in saliva as metabolites for the AnT determination. However, further investigations of this methodology should be conducted with a larger number of participants to assess LMT with saliva lactate in arm ergometer; determination not possible in our experiment.

One concludes that the determinations of the exercise intensities corresponding to the lactate minimum test in arm ergometer and cycle ergometer using sodium, potassium and lactate salivary concentrations seem not to be possible to estimate the lactademic LACmin in both ergometers based on the low regression coefficients obtained in the polynomial adjustments and in the weak correlation found between AnT determined with saliva metabolites and AnT determined with blood samples.

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