original article

Salivary and blood lactate kinetics in response to maximal workload on cycle ergometer

Cinética do lactato salivar e sanguíneo em resposta à potência máxima no cicloergômetro

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Abstract – The use of saliva may assist an emerging need for cost, time and invasiveness reduction, and special care involved in the collection of biomarkers, compared to blood tests. The aim of this study was to analyze the lactate kinetics in blood and saliva in response to graded cycle ergometer exercise. In a predictive correlational study, nine healthy male cyclists (24±2 years; 71.3±7.6kg; 170.9±4.7cm) were submitted to a graded exercise protocol, started at 10% of maximal workload (W_{MAX}). Blood and salivary lactate concentrations were measured every 3 minutes during exercise and at the 3rd, 6th, 9th, 15th, 30th and 60th minutes after exercise. To investigate the relationship between salivary and blood lactate, linear regression analysis was applied and the level of significance was set at p<0.05. There was a parallel evolution among the mean values of lactate measured in capillary blood and saliva with increasing workload ($R^2_{adjust.}$ = 0.93, p<0.001). It was concluded that although with different magnitudes, the lactate response during incremental exercise was similar between blood and saliva. Thus, the use of salivary lactate seems to be a noninvasive model for determining the blood lactate response to graded cycle ergometer exercise.

Key words: Lactic acid; Physical exertion; Salivary glands.

Resumo – *O emprego da saliva pode subsidiar uma necessidade emergente de redução de custos, invasividade, cuidados e tempo, comparado às análises sanguíneas. O objetivo do estudo foi analisar a cinética do lactato no sangue e saliva, em resposta ao exercício físico incremental em cicloergômetro. Em uma pesquisa correlacional preditiva, nove ciclistas saudáveis do sexo masculino (24±2 anos; 71.3±7.6kg; 170.9±4.7cm) foram submetidos a um protocolo de esforço progressivo, iniciado a 10% da carga máxima ($W_{MÁX}$), obtida previamente, com incremento de 10% a cada três minutos até a exaustão voluntária. A concentração de lactato sanguíneo e salivar foi medida durante o exercício e no 3º, 6º, 9º, 15º, 30º e 60º minutos pós-exercício. Para averiguar relações entre as curvas de lactato, foi realizada uma análise de regressão linear. Verificou-se uma evolução paralela entre os valores médios de lactato, medidos no sangue capilar e na saliva, com o aumento da carga de trabalho ($R^2_{ajust.}$=0.93; p<0.001). Conclui-se que, embora com magnitudes diferentes, a resposta lactacidêmica, durante o exercício incremental, foi similar entre o sangue e a saliva. Nesse sentido, o uso do lactato salivar demostrou ser um modelo não invasivo para a determinação do lactato sanguíneo em resposta ao exercício físico.

Palavras-chave: Ácido láctico. Esforço físico; Glândulas salivares.*

INTRODUCTION

Biochemical and physiological changes have been widely investigated in the area of human performance by biomarkers in blood[1,2], but also in other body fluids such as saliva[3,4]. Results support that substances present in saliva can be used to assess aerobic and anaerobic metabolisms[3,5,6], predominantly carried by blood. In this sense, alternative methodologies have emerged as a trend for using non-invasive techniques in anaerobic assessment[7], in maximum lactate steady state[8] and in minimum lactate tests[9] to verify relationships between blood and saliva during recovery from supramaximal exercises[10] and during long-distance running[11].

High correlations have been reported between salivary lactate concentration ($[Lac]_{saliva}$) and blood lactate concentration during short-duration and long-duration[11] exercise with progressive intensity[7,8,12]. However, to the present moment, studies have increased workload equally for individuals with different physical abilities, disregarding individual variability. In addition, saliva collection methods have differences that impact the way results are interpreted[13,14].

In the near future, the use of saliva will be able to support an emerging need to reduce costs, time and invasiveness, in addition to special care involved in the collection of biomarkers, compared to blood tests. Thus, using salivary lactate kinetics, compared to its blood counterpart, can contribute to assess quality, prescription and control of appropriate workloads to predominantly aerobic exercises for different age groups and special populations.

In addition, electromagnetic cycle ergometer has high applicability not only in assessments of the aerobic and anaerobic capacity, but also in training itself, especially for cyclists, with precise control of load, pedaling frequency and exercise time. To our knowledge, this research approach has not yet been applied to evidence differences in production and removal of lactate in response to individual progressive exercise on cycle ergometer. The aim of this study was to analyze the blood and salivary lactate kinetics in response to incremental exercise on cycle ergometer.

METHODOLOGICAL PROCEDURES

Subjects and ethical issues

Initially, 14 potential volunteers were recruited for the study. Of these, only nine met the inclusion/exclusion criteria and participated fully in the study. Subjects were cyclists, with practice time of 2.5±1.6 years and 4.6±0.8 hours of weekly cycling. Inclusion criteria were: 1) men, 2) age of 18-30 years and 3) training time of 4-6 hours per week. Exclusion criteria were: 1) reporting the use of ergogenic substances or any drug; 2) reporting musculoskeletal injury that prevented the completion of the cycling exercise; 3) do not reach minimum load of 250W in the maximal oxygen uptake test. Table 1 shows the physical characteristics of subjects. This study was approved by the local ethics committee. Subjects received prior instruction about procedures and

risks inherent of the study, as well as confidentiality of information to be acquired and voluntarily signed an informed consent form.

Table 1. Anthropometric and metabolic characterization of apparently healthy subjects (n = 9).

Variable	Mean ± SD	Min - Max
Age (years)	24±2	22 - 28
Body mass (kg)	71.3±7.6	61.5 - 81.2
Height (cm)	170.9±4.7	166.2 - 176.5
Σ3SF (mm)	52.9±18.7	26 - 84
$VO_{2peakabs}$ ($L \cdot min^{-1}$)	3.5±0.7	2.3 - 4.4
$VO_{2peakrel}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	47.7±9.0	33.4 - 65.6
W_{MAX} (W)	305.0±37.0	250 - 350
W_{MAXrel} ($W \cdot kg^{-1}$)	4.10±0.63	3.6 - 5.6

Σ3SF - Sum of chest, abdominal and thigh skin folds; VO2peakabs - Absolute peak oxygen uptake; VO2peakrel - Oxygen uptake relative to body mass; W_{MAX} - Maximum load W_{MAXrel} - Maximum load relative to body mass.

Study design

The subjects completed two sessions with 2-7 days interval between them: 1) anthropometric measurements and a test to determine maximum load (VO_{2peak} and W_{MAX}); 2) incremental exertion protocol with blood and saliva sample collections. All sessions were conducted in the morning from 8:30 a.m. to 11:30 a.m. in order to reduce circadian variations[14]. Subjects were instructed to: a) be well hydrated and feed 2-3h prior to data collection; b) abstain from alcohol, caffeine and moderate or vigorous exercise on the day before visits; c) brush teeth and do not use mouthwash; d) wear light clothes and appropriate footwear for physical exercise. All participants consumed their usual meals and performed their normal professional and recreational activities.

## Anthropometric measurements and maximum load determination

Body mass and height were measured in analog scale (Filizola, Brazil) and wooden stadiometer with accuracy of 0.1 kg and 0.1 cm, respectively. Body fat was estimated by the sum of chest, abdominal and thigh skin-fold measurements (Σ3SF) taken in triplicate in a rotational way through skinfold caliper (Lange Skinfold Caliper, Maryland, USA) with accuracy of 0.1mm. All variables were measured by a trained evaluator, according to standardized procedures[15].

Oxygen uptake (VO_2) was measured by a computerized metabolic analyzer (CPX/D Cortex, Germany) performing an incremental test in electromagnetic cycle ergometer (Cateye, Japan). The test was preceded by warm-up exercise performed on the same cycle ergometer for three minutes at 25W. The initial load was 50W, with increment of 25W every minute until volitional exhaustion or impossibility to maintain current load. Heart rate was continuously registered by means of an electrocardiograph with three derivations (Micromed, Brazil) using the PC Elite Software 3.0 (Micromed, Brazil). Respiratory variables were uninterruptedly measured and recorded every 20 seconds. The test was interrupted based on the demonstration of

at least two of the following criteria: (1) plateau or reduction in VO_2 with increasing load; (2) respiratory exchange ratio (RER) greater than or equal to 1.15; (3) range of 95% the maximum heart rate predicted by age (210-age)[16]. The highest VO_2 value before the test interruption was adopted as the VO_2 peak (VO_{2peak}). The highest load achieved was taken as the W_{MAX}, serving as basis for the calculation of the exertion protocol intensity. For this procedure, standardized instructions clarified before the test were adopted, so the volunteer was aware of all procedures and seat height was adjusted according to volunteer's height. Subjects were instructed about the test execution technique, being instructed to perform the tests with maximum effort possible and verbally encouraged during exercise.

Progressive exercise protocol

Prior to the implementation of the exercise protocol, subjects performed a warm-up exercise in electronic cycle ergometer (Cateye, Japan) for five minutes at 25W, followed by one minute of passive rest. Then, subjects were submitted to an incremental exercise on a cycle ergometer, starting with proportional load of 10% W_{MAX} (in Watts) with increment of 10% every three minutes until voluntary exhaustion or impossibility to maintain current workload. Volunteers ingested 400-500mL of water 30 minutes before the effort and hydration during exercise or recovery was not allowed. Wherever possible, the volunteer was asked for bladder emptying before beginning the protocol. Blood and saliva samples were collected at rest, at the end of each stage and at the 3rd, 6th, 9th, 15th, 30th and 60th minutes after exercise (Figure 1), with individual seated. The time between the collection of blood and saliva samples was less than 40 seconds.
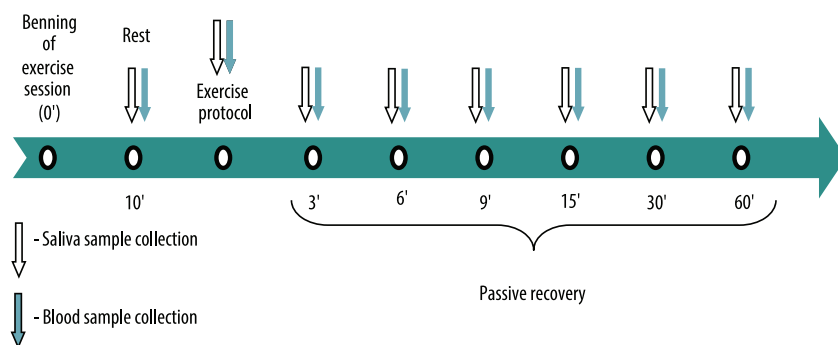
Figure 1. Experimental design for the second session as a function of time. REP - rest; REC - recovery. Collections during the exercise protocol were performed at the end of each stage (up to 10 collections).

Lactate measurement and analysis

For blood collection, the palmar surface of the distal phalanx (digital) was punctured with a disposable lancet (Softclix, Germany) and blood samples were collected (25μL) with pre-calibrated heparinized capillary and transferred to eppendorf tubes containing 50μL of 1% sodium fluoride (NaF).

Before each saliva collection, the mouth was previously washed with deionized water to prevent contamination of cell debris and other materials,

discarding in appropriate container, according to methods described by Pilardeau et al.[17]. Saliva was collected with Salivettes® kits (Sarstedt, Germany), in which cotton was chewed as a form of mechanical stimulation, 30 seconds before the end of each stage. To avoid contamination of salivary lactate samples with sweat, frequent cleaning of the volunteers' face was performed with paper towels.

The saliva volume was indirectly determined by double weighing on an analytical scale (Mark 210A, Bel Engineering, Italy), considering only the tube and the tube + saliva[12,13]. Saliva was immediately stored at 4°C (≤3h)[18] and centrifuged at 2200rpm (Centribio, Brazil) for two minutes. For lactate measurement, 25μL were injected into electroenzymatic lactimeter (1500 Sport, YSI Inc., USA) with accuracy of 0.1mM and the results were expressed in mM.

Data analysis

Normal distribution was checked by the Shapiro-Wilk test ($p < 0.05$). Data were reported as mean, standard deviation and maximum and minimum values. After dispersion analysis, linear regression was used to verify relationships between blood and salivary lactate during exercise. Saliva volume (V_{sal}) was compared between rest (Basal) and the other stages using analysis of variance (ANOVA) for repeated measurements, with correction of Greenhouse-Geisser and simple contrast to the first reference category (Basal). Furthermore, the percentage instantaneous variation rate (Δ%) between stages was calculated by the equation: $\Delta\% = 100 \times ([\ ]_{initial} - [\ ]_{final}) / [\ ]_{inicial}$, where [ ] is the lactate concentration. Analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, IL, USA), and the level of significance was set at $p < 0.05$.

RESULTS

Eight subjects reached the stage corresponding to load greater than or equal to 80% W_{MAX} in incremental exercise protocol. The average [Lac]$_{saliva}$ values during exercise ranged from 6 to 20% compared to blood. There was a parallel evolution between mean lactate values measured in capillary blood and saliva in response to increased workload (Figure 2).

This behavior is justified by the simple linear regression analysis (Figure 3), with statistically significant parameters and low standard error of estimate at 95% confidence level. The average lactate concentration in saliva can explain 93.4% of blood lactate variation during exercise, illustrated by the determination coefficient (R^2_{adjust}). The saliva volume did not change significantly during exercise or recovery, $F_{(2, 63)} = 3.41$; $p = 0.054$, indicating that the lactate concentrations in saliva were independent of volume.
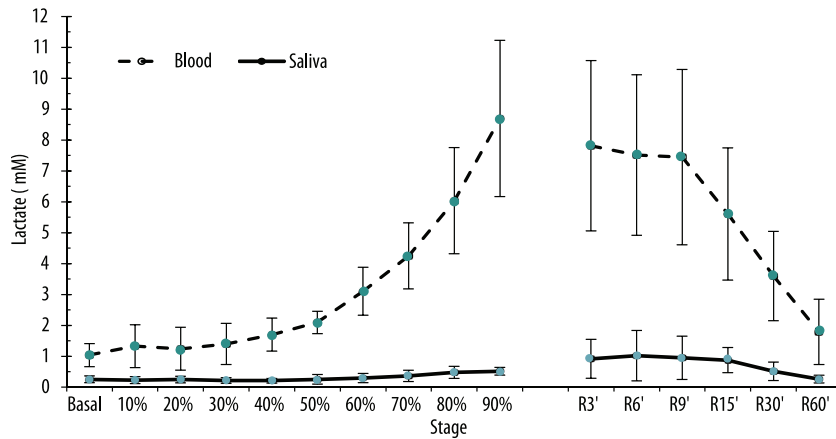
Figure 2. Average salivary and blood lactate concentration of individuals submitted to incremental exercise on a cycle ergometer (stages based on the percentage of maximum power) and during recovery. Values were expressed as mean and standard deviation (n = 9).
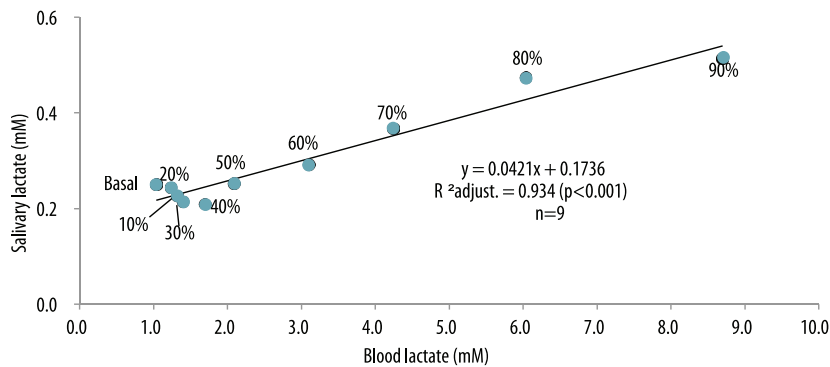
Figure 3. Adjusted linear model of salivary lactate with blood lactate during graded cycle ergometer exercise. Mean values of the resting stage at 90% WMAX, $F_{(1, 8)} = 127.7$; $p < 0.001$.

In the analysis of the percentage variation rate (Δ%), it was observed that the blood and salivary lactate curves were similar, although with different magnitudes (Figure 4). This phenomenon is very evident if we consider an approximate delay of 6 minutes from the appearance of lactate in the saliva in relation to blood, especially up to the 24th minute. Blood variation rate presents a major peak at the 18th minute (48.2%). In saliva, increases in two subsequent time points, 21st (25.6%) and 24th (29.6%) minutes stand out. In recovery, the blood lactate Δ% tends to rise up to the 9th minute and in saliva, there is reduction over time, with a slight peak at the 15th minute.
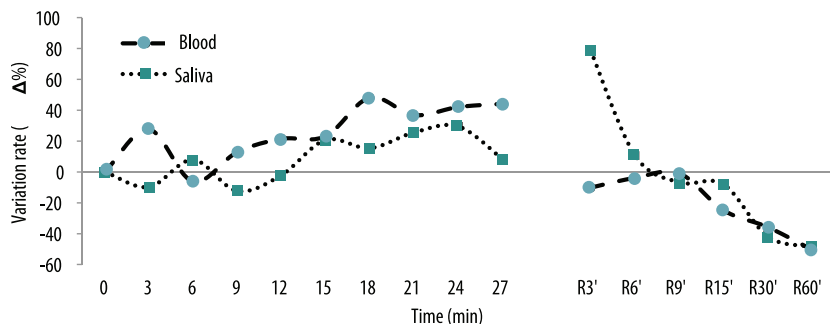
Figure 4. Lactate kinetics: percentage variation rate in blood and saliva as a function of time during incremental exercise in cycle ergometer and passive recovery (n=9).

DISCUSSION

The use of saliva has emerged as an interesting tool for biochemical, metabolic and functional diagnosis in different populations[3,5,6,19]. However, there seems to be a lack of studies using salivary lactate for monitoring stress induced by exercise. This gap may refer to a need for further studies in order to characterize a new area of research or represent little success in contexts where it has been used.

The main finding of this study showed similar responses in salivary and blood lactate concentration curves during graded cycle ergometer exercise and recovery. Thus, the linear increase in $[Lac]_{saliva}$ with increasing workload suggests a non-invasive model to measure physical exercise intensity. However, a delay in the onset of salivary lactate compared to blood was observed. Advantages of saliva samples compared to capillary or venous blood samples have been reported in different studies[6-8]. However, our proposal has made important methodological adjustments over current studies[10-12,20,21] regarding load prescription, increasing into deciles of maximum power (W); use of salivette° saliva collector kit; and post-exercise analysis in six moments and in recovery.

The standardization of load values in $\%W_{MAX}$ for subject was considered relevant because it reinforces the principle of biological individuality and allows interpretations and objective comparisons. Perez et al.[8] reported a more significant relationship when maximal lactate steady-state was expressed by load and not by VO_{2max}, while Segura et al.[7], in incremental protocol, did not present their results as relative load using progressions of 25W each three minutes. Thus, this study found that individuals with different physical fitness levels performed exercises based on a previously prescribed load.

The use of the salivette° kit enhanced the saliva collection logistics regarding quantity, easy handling and safe storage of samples. To date, few studies[20,22,23] have used this type of collector, but rather, the saliva expectoration collection technique reported has been used in some studies that analyzed salivary lactate as a function of exercise[7-10,12].

Moreover, no previous studies have made more blood and saliva collections in recovery than ours. This procedure allows for different forms of inference on performance and the detection of difference metabolic response (acute) to exercise. Segura et al.[7] performed three observations after incremental exercise (3, 6 and 9 minutes). Karatosun et al.[10] analyzed the salivary lactate shortly after anaerobic exercise and also in the fifth and fifteenth minutes. The one closest to the number of collections in recovery was the work of Ohkuwa et al.[12], with five measures.

The salivary lactate concentration was lower than in blood, corresponding to approximately 6-20% of that observed in blood samples obtained at the same time from finger digital. Salivary lactate seems to be a suitable parameter to estimate blood lactate at increasing exercise intensities, since significant determination coefficient values and low estimate of error have

been reported, characterizing it as a predictive model of low invasiveness, from the method applied.

In addition to the linear growth both of salivary and blood lactate with increased load, the blood variation rate clarifies an important peak at the 18th minute ($\approx$60% W_{MAX}), in which it could be inferred that the stage prior to this showed a consistent point for the lactate threshold[24]. In the saliva, the substantial increase occurred after the 21st minute ($\approx$70% W_{MAX}) seems to represent better this threshold, also considering the stage that preceded it. In practice, if on visual inspection, there is no clear inflection point in the lactate curve with increasing intensity, particularly in saliva, which shows discrete peaks, the graphical analysis of the percentage variation rate can be used.

The blood lactate recovery curve values of this study are consistent with literature data[25,26]. Rimaud et al.[27] and van Hall[28] report that high lactate concentrations in the recovery can be explained by the high production of this metabolite during exercise by a decrease of the removal rate or by a combination of these two possibilities.

There is stabilization in blood lactate levels up to the ninth minute of recovery, followed by a sharp decrease, approaching baseline values. Moreover, this response is not found in saliva, since it extends up to the 15th minute, when there is a continuous decrease of this metabolite. In this sense, an interpretation of the Δ% of $[Lac]_{saliva}$ elucidates a delay in its onset in relation to blood of approximately six minutes during exercise. The fact that other tissues participate in the lactate turnover[28], as well as the integrative effect of the hemodynamic and neuroendocrine response in saliva flow and components[3], supports the difference in time of their appearance in saliva.

The salivary volume of subjects in this study did not change significantly during incremental exercise or recovery. In this context, there is evidence that the concentrations of lactate and other electrolytes in saliva appear to be volume-independent. Due to the fact that the cotton used is saliva collector is of high absorption and stimulates salivary flow[29], possible differences in volume between stages and recovery could be minimized, allowing good conditions for the analysis of components and added quality in methodological issues of saliva use in experiments in the area of exercise.

The effects of training and influence of exercise time have not yet been studied using salivary lactate analysis. Such gaps are also found in populations of extreme ages (children and elderly) and different movement patterns (swimming, rowing, team sports in general). In this perspective, it is plausible to support proposals with the use of salivary lactate as a parameter to support the prescription of exercise intensity[7] in addition to advantages related to saliva collection methods[13].

The main limitations of this study deal with greater control over eating and oral health variables (which were only informative and self-reported), due to the possible variability in saliva properties. Thus, parallel analyses of other salivary components, whose changes may be in line with exercise

intensity, are suggested. In this sense, the discussions presented in the text were limited to present a model for the use of salivary lactate as a trend in science to meet the needs of everyday life, as is the case of less invasive and lower-cost alternatives.

CONCLUSION

Although with different magnitudes, the lactate response during exercise individually controlled by W_{MAX} percentages in cycle ergometer and in passive recovery was similar between blood and saliva; however, a delay approximate of six minutes in the appearance of lactate concentration in saliva compared to blood should be considered. In this context, the use of salivary lactate seems to be a noninvasive model for the determination blood lactate in response to graded cycle ergometer exercise.

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