CAN SALIVA REPLACE BLOOD IN MONITORING AND TRAINING CONTROL?

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Original scientific paper

Abstract

A reliable indicator of metabolic changes during physical activity is body fluids acidosis. In practice, blood lactate levels have been used most often. The aim of this study is to examine if saliva, which is sampled quickly and painlessly, can replace blood whose sampling is invasive and unpleasant. Running on treadmill with progressive load has been applied on a sample of 41 young men (13 athletes and 28 students). After every 3 minutes and during recovery blood lactates and pH of the saliva were measured. The students reached maximum values of blood lactates earlier than the athletes. Saliva pH in both groups changed significantly only during the highest running speed. These changes emerged later than blood lactates changes. Blood lactates remained significantly increased until the end of recovery, while saliva pH returned to the initial values. Results of this study show that saliva pH is not reliable predictor of blood lactates and cannot be used to track metabolic changes during physical activity.

Key words*: functional diagnostics, lactates, pH, progressive load*

Introduction

Physical activity of high intensity causes significant changes in the anaerobic metabolism. A reliable indicator of these changes is body fluids acidosis, meaning the increase of hydrogen-ion concentration (pH) in the blood, saliva, urine, and sweat. Lactates concentration in the blood is the most frequently used data regarding acidosis (pH decrease), which occurs during physical activity. Diagnostic practice has shown BL is reliable tool for monitoring and training control (Harnish et al., 2001; Pyne et al., 2001; Schabort et al., 2000; Tékus et al., 2012). Anaerobic threshold (AT) can be determined using BL, which is a good load level indicator in sports and fitness (Billat et al., 2003; Faude et al., 2009; Macintosh et al., 2002). AT corresponds to the maximum level exercise intensity, where the registered balance between the production and removal of blood lactates (BL) for most healthy persons is 4 mmol/L, with the range of 3 to 5.5 mmol/L (Heck et al., 1985; Mann et al., 2013).

The blood sample for lactate concentration analysis is taking by invasive procedure which is unpleasant and painful for many athletes. The body fluids communicate interchangeably, meaning that hydrogen-ion concentration is rising in all bodily fluids by decreasing their pH. Some researchers tested whether AT, instead of BL, can determined based on the metabolites present in the saliva (Chicarro et al., 1994; Chicarro et al., 1995; Pérez et al., 1999; Segura et al., 1996) or in sweat (Green et al., 2004). In this way, they have attempted to avoid the unpleasantness caused by invasive procedure of blood sampling. The registered changes did not have the same volume and the same dynamics in all body fluids, but they have indicated a certain correlation. The most studies that simultaneously tracked biochemical changes in the blood and the saliva caused by physical activity have reached similar conclusions (Santos et al., 2006; Tékus et al., 2012; Zagatto et al., 2004).

Apart from physical activity, saliva acidosis is caused by a range of other external factors (food, drinks, cigarettes, stress). Their control is often impossible and it is difficult to determine precisely which factor has stronger or weaker influence on the saliva pH changes. The most useful data for monitoring and training control are saliva lactates (SL). Laboratory analyses of saliva sample taken from the athletes mouths during activity are very complex (Santos et al., 2006; Tékus et al., 2012; Zagatto et al., 2004). Several hours is necessary to extract SL. In practice, the saliva samples are usually frozen and used a few hours (or days) after physical activity. So, the SL data cannot get in real time as BL.

Blood is a much faster source of information compared to saliva, but obtaining by invasive (painful) procedures. The BL measuring procedure is unpleasant, and the SL analysis process lasts a long time. In our study, saliva pH (SpH) was used instead saliva lactates (SL). SpH is obtaining very quickly by painless procedure. The SpH data were compared to the BL data sampled at the same time. The aim of this study was to analyse relation between SpH and BL, and answer the question – can saliva use as an alternative informative source about metabolic changes during physical activity?

Methods

The experiment was approved by the ethics committee of the Faculty of Sport and Tourism (protocol number: EN-02/2017). Prior to starting the test and blood and saliva sampling, the protocol was explained in detail. The participants have been familiarized with all the possible unpleasantness and they signed a consent of voluntary accession to the test. The study was performed in accordance with the Declaration of Helsinki.

Sample

The sample is formed by 41 students of the Faculty of Sports and tourism, ages 21-27, who voluntary applied for the research. All participants are healthy males, regularly physically active, with good work ability. The participants approached to the test in the morning hours after a night of sleep, and did not consume any foods or drinks at least 2 hours before the test. All participants were high motivated for the research.

The sample is divided into two groups (athletes and students) based on the anaerobic threshold (AT). The value of 4mmol/L was used to determine AT because in the most previous studies (Billat et al., 2003; Faude et al., 2009; Macintosh et al., 2002; Mann et al., 2013) it was recognized as a scree point where the BL production speed becomes significantly higher than its removal speed. The athletes (N=13) reached 4mmol/L value at high speeds (8 participants at 14 km/h, while five did not reach AT until the end of test), and the students (N=28) at lower speeds (14 participants at speed of 12 km/h, 8 at 10 km/h, and 6 already at 8 km/h). The interview revealed that athletes train and compete regularly for several years, while the students exercise only recreationally.

Variables and test protocol

The test-protocol was conducted using Trackmaster JAS treadmill, model TMX425C. Only one examinee was on the track during the test. The six measurers monitored him simultaneously: the one controlled the track speed, one followed the heart rate (HR), the two sampled the blood and read BL, and the two sampled saliva and determined SpH. The statistician entered the data into a SPSS base just after measurement.

The HR was recorded during the whole protocol using Polar pulse meter and holter monitor, FT60 type. Calibrated Nova Biomedical 40828 analyser was used to determine the BL. The samples of capillary blood were taken from the finger by sterile Owen Mumford single use safety lancet. In order to ensure fresh blood during every sampling and to avoid problems with coagulation and damaging of previous pinch point spot, subsequent sampling was always done from another finger (whether left or right hand). BL value automatically displayed on analyser with the precision of 0.1 mmol/L. The Ahlstrom Munktell pH litmus paper was used for the saliva sampling with precision of 0.3 scalar units. Saliva sampled from the mouth floor (zone of sublingual fold) by litmus paper direct contact. SpH was determined by comparing the color obtained with the closest shade on the control range.

The test protocol lasted for 30 minutes in total. HR, BL and SpH were measured just before the treadmill work. Three minute warm-up by walking at the speed of 6 km/h. The track speed increased to 8 km/h and the examinee started a three-minute running. The track speed progressively increased 2 km/h every 3 minutes. Each examinee was running 12 minutes in total during the test: 4 periods of 3 minutes with progressive speed increase (8, 10, 12 and 14 km/h). The blood and saliva sampled every three minute and BL and SpH were registered. The examinee stopped to run during blood and saliva sampling for safety and hold to the handrails. Activity break lasted for about 30 seconds. The examinee left the treadmill when finished the test and sat on a chair. During the rest period BL and SpH were measured two more times: 3 minutes and 10 minutes after sitting down.

Statistical analysis

The descriptive parameters (Mean and Standard Deviation) were calculated for each variable. The mixed between-within subjects ANOVA (Tabachnick and Fidell, 2013) was applied to test influence of running intensity (load level) and participants physical work capacity (PWC) to the differences between the mean. The Pearson's coefficient was used to determine correlation between BL and SpH. All the conclusions were realized on the 0.05 level of significance ($p<0.05$). The IBM SPSS v.21 application was used for complete statistical analysis (License Stats Prem: 761b17dcfd1bf20da576 by Hearne software).

Results

The data registered during the test-protocol indicate that progressive overload on treadmill lead to the expected metabolic changes: BL increased (Figure 1) and SpH decreased (Figure 2). During recovery, empirical values of both variables have the reversed direction: BL decreased while SpH increased. The tempo of these changes was different; BL had higher increase. Its maximum values were registered during the highest load (the speed of 14 km/h). The students had 4-times and the athletes 5-times higher BL compared to pre-test values. At the same time, much lower changes were recorded for SpH. Initial values of acidosis did not change more than 0.2 scalar units.

Heart rate in both groups of examinees (athletes and students) showed a similar tendency during progressive load and recovery (Figure 3). The athletes had a significantly lower values at each protocol point. It was expected HR loses the linear and receives an exponential AT level growth, considering the applied progressive load. However, this did not happen. The running interruptions after every three minutes due to blood and saliva sampling are logical explanation for this. Practically, type of applied load was intermittent and not typical continual.

The changes trend of metabolic variables (BL and SpH) had similar dynamics in both groups (Table 1). However, the ANOVA results (Table 2) indicated that the athletes had statistical significantly lower BL and SpH than the students during the whole test-protocol. The run intensity and participant's PWC had a significant impact on the changes of anaerobic metabolism only separately, but not jointly.

The correlation between BL and SpH was conducted separately for all characteristic protocol points. The results obtained reveal significant correlation only for pre-test data (r=-0.336; p=0.032). All other coefficients are very low and show that there no significant correlation between BL and SpH at any test-protocol point (for 8 km/h speed running r=0.219 p=0.169; for 10 km/h r=0.108 p=0.501; for 12 km/h r=-0.096 p=0.550; for 14 km/h r=0.148, p=0.355; at 3rd minute recovery r=-0.165 p=0.302; at 10th minute recovery r=0.191 p=0.571).

Figure 1. The changes of Blood Lactates during the test on a treadmill; R.3 = 3rd minute recovery; R.10 = 10th minute recovery.

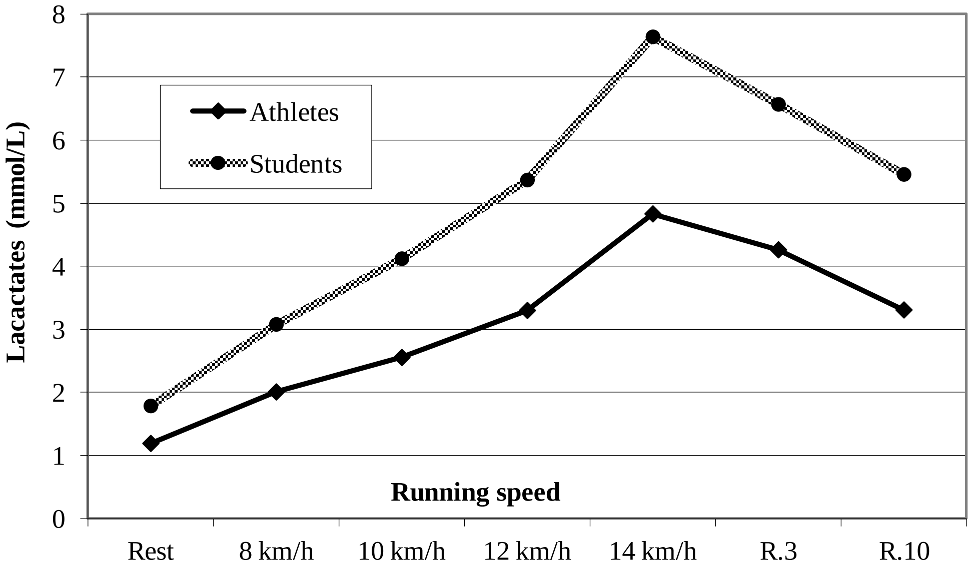

Figure 2. The changes of Saliva pH during the test on a treadmill; R.3 = 3rd minute recovery; R.10 = 10th minute recovery.

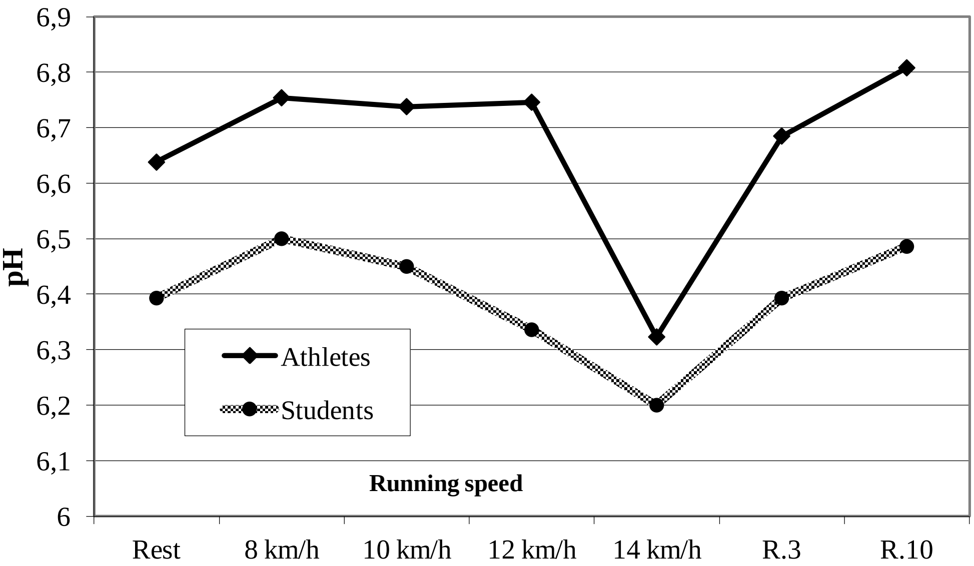

Figure 3. The changes of Heart Rate during the test on a treadmill; WU = Warm Up; R.3 = 3rd minute recovery; R.10 = 10th minute recovery.

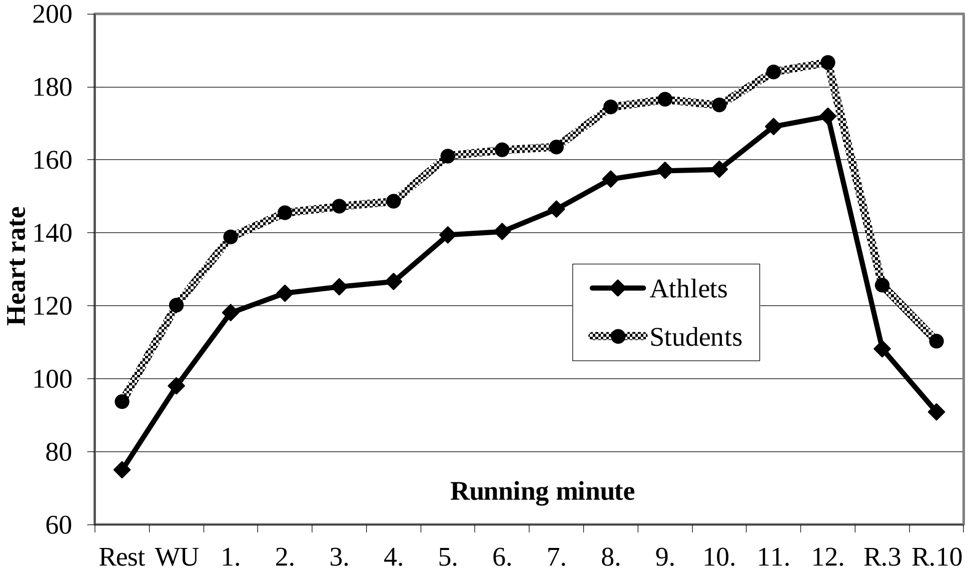

Table 1. Descriptives for Blood Lactates (mmol/L) and Saliva pH during the test on a treadmill of the athletes and the students.

			Blood lactates		**pH**	
Intensity (Running speed)	**Group**	N	**Mean**	SD*	**Mean**	SD*
Inactivity (pre-test)	Athletes	13	**1.19**	0.333	**6.64**	0.302
	Students	28	**1.79**	0.637	**6.39**	0.258
8 km/h	Athletes	13	**2.01**	0.578	**6.75**	0.151
	Students	28	**3.08**	0.979	**6.50**	0.334
10 km/h	Athletes	13	**2.55**	0.847	**6.74**	0.166
	Students	28	**4.12**	1.486	**6.45**	0.350
12 km/h	Athletes	13	**3.30**	1.523	**6.75**	0.156
	Students	28	**5.37**	1.806	**6.34**	0.326
14 km/h	Athletes	13	**4.83**	2.285	**6.32**	0.573
	Students	28	**7.64**	2.332	**6.20**	0.348
3rd minute recovery	Athletes	13	**4.26**	1.873	**6.69**	0.212
	Students	28	**6.57**	2.079	**6.39**	0.386
10th minute recovery	Athletes	13	**3.31**	1.427	**6.81**	0.263
	Students	28	**5.46**	2.147	**6.49**	0.417

* SD = Standard Deviation

Table 2. ANOVA parameters obtained by evaluating the impact of run intensity and participants physical work capacity (PWC) on the metabolic changes caused by progressive load.

Variables Impact	Wilks' Lambda	F	**p**	Partial Eta Squared
Blood Laktates				
Run Intensity + PWC	0.812	1.309	0.280	0.188
Run Intensity	0.167	28.345	0.000	0.833
PWC		18.898	0.000	0.326
Saliva pH				
Ran Intensity + PWC	0.775	1.643	0.166	0.225
Run Intensity	0.507	5.515	0.000	0.493
PWC		12.451	0.001	0.242

Discussion

The SpH Mean did not exceed the theoretical range (5.45 to 7.8) interpreted in previous studies as daily average for most people (Cook, 2008; Tyler, 2014) at any measuring point. The SpH of most subjects returned to approximately baseline after 3 minutes of recovery. At the same time, BL values remained increased even after a 10-minute recovery, particularly in group of students. These were the first indicators suggesting a negative response to the initial question regarding the saliva use for changes monitoring of anaerobic metabolism during physical activity in real time.

There was no significant correlation between BL and SpH at any test-protocol point, except pre-test. A significant correlation between SpH and BL determined only during inactivity and it is probably consequence of diet and activities control of participants before the test. Avoiding anaerobic training 24 hours and the absence of food and drink two hours prior to the testing decreased the possibility of body acidosis. Previous studies (Cook, 2008; Santos et al., 2006; Tyler, 2014) warn that consuming coffee, cigarettes or even water is sufficient to create measurable changes in SpH. Without a strict control of these influences, every application of saliva in monitoring of metabolic changes during physical activity would be pointless. In everyday practice, the trainers usually cannot control those influences. These data confirmed a negative answer to the initial study question and indicated the saliva cannot be recommended to sports practice as a reliable alternative information source of metabolic changes during physical activity. Based on this research, it conclude that saliva cannot replace the blood in monitoring and training control.

A more serious saliva acidosis increase recorded only during the highest intensity, while BL changed significantly from the beginning of the protocol and grew steadily from point to point of measurement. Comparing with BL, the SpH changes were notably slower and less pronounced. In our study, pH changes occurred 5-6 minutes later than BL changes. Almost identical delay time of SpH compared to BL in some athletes (bicyclists, runners, swimmers, and football players) determined in previous studies (Bretz and Carrilho, 2013; Karatosun and Baydar, 2005; Oliveira et. al, 2015; Tékus et al., 2012; Zagatto et al., 2004). It is significantly shorter time than delay of saliva lactates monitored during the high intensity running, which was over 30 minutes in some marathon runners (Santos et al., 2006).

The doubt regarding the applicability of information from saliva is supported by observations of researchers who sampled it in this study. It was noted the litmus paper is not sufficiently precise and not ensure the necessary data discrimination. They reported the problem of recognizing the corresponding shade in the control spectre which influences directly the objectivity of the measurement. In our study, the same person always determined the sample colour which decreased the error possibility.

The modern pH meters enable higher precision and more objectivity hydrogen-ion concentration measurement. These devices for saliva analysis is require the sampling of a larger fluids quantity in which an electrode is submerging. The most user manuals for pH meters recommend the 4 cm minimum fluid depth which is impossible with saliva because its quantity in the mouth is insufficient. Some researchers attempted to identify the changes in the saliva during physical activity by infrared spectroscopy (Khaustova et al., 2010). This procedure also cannot provide information in real time, even though a small saliva quantity uses, because it requires multi hours laboratory analysis.

Conclusion

The saliva usability as an alternative information source of metabolic changes caused by progressive load did not prove. The blood is still the most reliable body fluid for real-time exercise monitoring. Lactate concentration is growing in the blood from the exercise beginning, while saliva pH changes significantly only at the highest working load. Saliva pH changes occur 5-6 minutes later than blood lactates changes. A significant correlation between SpH and BL is possible only at the body rest.

Acknowledgments

This work was supported by the Provincial Secretariat for Higher Education and Scientific Research of Autonomous Province of Vojvodina (grant number: 142-451-2612/2017-01/02).

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Received: 12.06.2020.
Accepted: 01.11.2020.
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