

COMPARISON OF BLOOD AND SALIVA LACTATE LEVEL AFTER MAXIMUM INTENSITY EXERCISE*

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Several studies have described high correlation of salivary and blood lactate level during exercise. Measuring the effectiveness and intensity of training, lactate concentration in blood, and lately in saliva are used.

The aim of our study was to evaluate the correlation between the concentration and timing of salivary and blood lactate level in endurance athletes and non-athletes after a maximal treadmill test, and to identify physiological and biochemical factors affecting these lactate levels.

Sixteen volunteers (8 athletes and 8 non-athletes) performed maximal intensity (Astrand) treadmill test. Anthropometric characteristics, body composition and physiological parameters (heart rate, RR-variability) were measured in both studied groups. Blood and whole saliva samples were collected before and 1, 4, 8, 12, 15, 20 min after the exercise test. Lactate level changes were monitored in the two groups and two lactate peaks were registered at different timeperiods in athletes. We found significant correlation between several measured parameters (salivary lactate – total body water, salivary lactate – RR-variability, maximal salivary lactate – maximal heart rate during exercise, salivary- and blood lactate –1 min after exercise test). Stronger correlation was noted between salivary lactate and blood lactate in athletes, than in controls.

Keywords: Salivary lactate – blood lactate – exercise – endurance athletes – RR-variability

INTRODUCTION

The concentration of certain molecules was proved to be significantly related in blood and in saliva [10] of healthy and unhealthy subjects, too. Many publications have reported exercise-induced changes in saliva composition [4, 7]. Physical activity modifies the amount of some salivary factors [6] and hormones (cortisol; [16]), electrolytes (Na⁺, K⁺, Mg²⁺; [11, 22]), enzymes (α -amylase; [5]) and immunoglobulins (SIgA; [1, 13]).

Since salivary lactate (SL) is a useful molecule to measure the effectiveness of training, it has been investigated by many authors in different exercise models [14].

*Dedicated to Professor József Hátori on the occasion of his 80th birthday.

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First Mendez and his colleagues found significant correlation between SL and blood lactate (BL) concentration during exercise, in an other research also a high correlation was reported in BL content and SL before and during a long distance race (30 km; [23]). BL and SL following a 400 m and 3000 m run in sprinters and long-distance runners was also measured [15], and a significant relationship was found between the peak SL and BL level after 400 m run. A maximal graded exercise test on cycle and arm ergometer was performed by amateur athletes, and it was concluded that SL, sodium and potassium concentrations could not be used as determinants of the anaerobic threshold [29].

Santos also reported that there isn't enough information on the changes in SL after exercise, concerning the timeframe of peak lactate concentration and clearance in blood and in salivary gland [23]. In an earlier study it was demonstrated that the elevation of SL level appears potentially delayed compared to blood samples [21]. Autonomic nervous system control of the heart is often analyzed with the variability of the R-R intervals and reported as Heart Rate Variability (HRV; [24]). Fluctuations of HRV show changes in cardiovascular control via autonomic nerves. The changes above 0.15 Hz (high frequency, HF) corresponding to respiratory sinus arrhythmia and are mediated by vagal nerve control only (for review see, [18]). Fluctuations under 0.15 Hz (low frequency, LF 0.04–0.15 Hz) are due to arterial pressure control, caused by cardiac vagal and sympathetic nerve innervation, too. HRV under 0.04 Hz is considered as the effect of peripheral vasomotor regulation. This way the quantitative analysis of HRV reflects the autonomic control of the studied subject. The ratio of LF/HF mirrors the changes in sympatho-vagal interaction [17].

The aim of our study was (i) to describe the correlation between the concentration and timing of SL and BL level in endurance athletes and non-athletes after a maximal treadmill test and (ii) to identify the effect of hydration status, RR-variability in changes of lactate content in saliva.

MATERIALS AND METHODS

Sixteen healthy volunteers (8 male, 8 female) participated in this research. Endurance athletes and non-athletes as control subjects (mean age: 22.80 ± 3.18 years) were also in the studied group. They are all healthy, nonsmoker university students. Athletes had regular trainings at least 7.5 h/week.

Collection of blood and saliva

Samples were collected in the morning (9.00–12.00 a.m.) since high saliva secretion can be measured during this period [12]. Subjects have been instructed not to eat and drink, except (non-carbonated) mineral water two hours before sampling. Five min before collection of saliva, mouth was rinsed with water. Whole saliva samples were collected into Eppendorf tubes by spitting before and 1, 4, 8, 12, 15, 20 min after the

exercise test without any chemical stimulation. Salivary samples were stored at -20°C until lactate measuring. Blood samples were taken from fingertips at the same times.

Exercise testing and RR-variability

R-R interval was registered according to Baynard's method [3] with RS-800 HR monitor (Polar Electro, Kempele, Finland) and an electrode transmitter belt. Subjects rested in seated position for 5 min. Right after the resting period RR-data were recorded for 5 min. Then each subject performed a maximal Astrand treadmill test [2] meanwhile heart rate (HR) data were registered. The speed of treadmill (Trackmaster TMX-425, Full Vision Inc., Newton, USA) was adjusted to 8 km/h at all stages of the protocol and the incline at the beginning of the test was 0% for 3 min and it was increased by 2.5% every two min. The test was stopped, when the participant was unable to continue. After the test, RR-interval was continuously recorded for another 5 mins. The results were processed with Polar Pro Trainer 5 software (Polar Electro, Kempele, Finland). Recorded data were the mean RR intervals, the number of heart beats (NHB), very low frequency (VLF, <0.04 Hz), low frequency (LF, 0.04 Hz–0.15 Hz), high frequency (HF, 0.15 Hz–0.40 Hz) and the ratio of LF/HF.

Body composition and total body water (TBW)

Basic anthropometric parameters (weight, height, BMI) were registered in both groups. Body composition and total body water were measured by bioelectrical impedance analysis (Tanita BC-420 MA; Tanita, Tokyo, Japan) before the exercise test.

Sample analysis

Spectrophotometric analyses were applied to measure lactate content in saliva by using LDH to oxidize lactate in the presence of NAD^{+} to pyruvate by monitoring NAD^{+} reduction at 340 nm. Three replicate samples were taken in each measurement [19]. BL was determined with a portable lactate analyzer (Lactate Scout, SensLab, Leipzig, Germany) immediately after collection. BL and SL concentration were expressed in mM/L.

Statistical analysis

Results of measurements were evaluated by one-way analysis of variance (ANOVA) and Pearson test to determine correlation between lactate concentration in blood and saliva. Values are reported as mean $\pm$ SD (standard deviation), or mean $\pm$ SEM (standard error of mean). The level of significance was adopted at $p < 0.05$.

RESULTS

Anthropometric data of measured subjects are summarized in Table 1. There are no significant differences in the measured parameters between athletes and non-athletes (controls). In fat free mass the control group is very similar to the athletes. However, we found differences in body fat (Table 1) between the groups but they did not differ in other essential characteristics.

The SL and BL concentrations were measured after a maximal treadmill test in two groups (endurance athletes and non-athletes). Two lactate peaks were detected in each athlete subject in saliva after the test protocol (Fig. 1). They were divided into two

Table 1
The anthropometric characteristics of the two studied groups

	Athletes		Controls	
	Female (n = 4)	Male (n = 4)	Female (n = 4)	Male (n = 4)
Age (years)	25.15±1.46	20.66±1.76	22.10±1.16	23.31±1.57
Weight (kg)	54.3±1.81	71.25±4.98	66.63±5.28	67.33±5.91
Height (m)	1.65±0.01	1.85±0.02	1.69±0.03	1.79±0.01
Fat mass (%)	14.03±1.26	4.70±1.12	27.98±3.15	10.65±3.78
Fat-free mass (kg)	46.85±0.98	68.13±4.32	47.68±1.57	60.08±2.67
BMI (kg/m ²)	19.95±0.66	20.73±1.14	23.57±2.34	21.07±1.93
Training volume (hours/week)	11.13±2.68	19.25±4.23	0.75±0.75	2.63±1.55

Data are mean ±SEM.

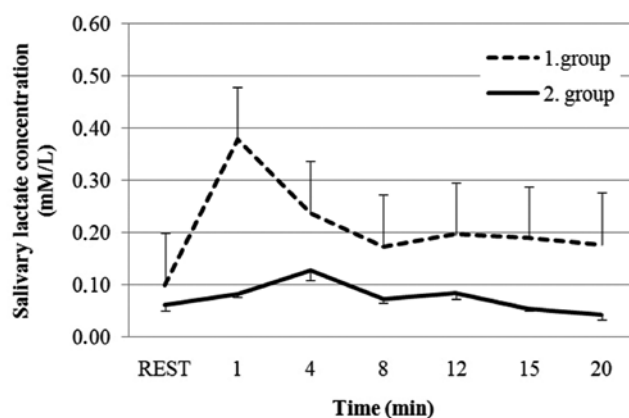


Fig. 1. Salivary lactate concentration in the athlete groups. Subjects were grouped by lactate concentration topography after the exercise test. Data are mean ±SEM

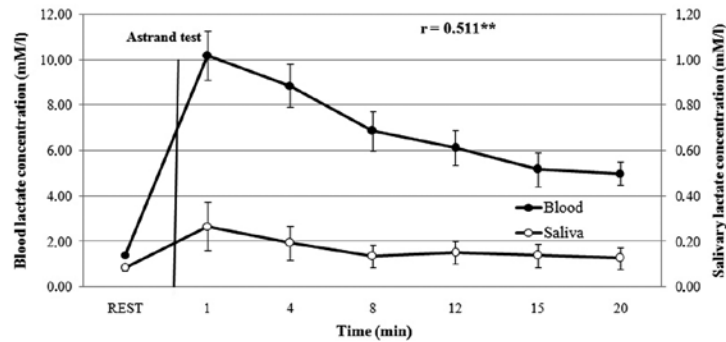


Fig. 2. Salivary and blood lactate concentration is significantly correlated in athletes. Values are mean $\pm$ SEM

Table 2

Blood and salivary lactate concentration in athletes and non-athletes before and after exercise

Recovery (min)	Athletes		Controls	
	Blood (mM/L)	Saliva (mM/L)	Blood (mM/L)	Saliva (mM/L)
Rest	1.41 $\pm$ 0.08	0.09 $\pm$ 0.01	1.34 $\pm$ 0.13	0.11 $\pm$ 0.02
1. min	10.20 $\pm$ 1.08	0.27 $\pm$ 0.11	11.20 $\pm$ 0.63	0.34 $\pm$ 0.08
4. min	8.86 $\pm$ 0.96	0.19 $\pm$ 0.07	10.81 $\pm$ 0.53	0.33 $\pm$ 0.04
8. min	6.86 $\pm$ 0.86	0.13 $\pm$ 0.05	10.03 $\pm$ 0.75	0.29 $\pm$ 0.06
12. min	6.11 $\pm$ 0.77	0.15 $\pm$ 0.05	9.31 $\pm$ 0.92	0.32 $\pm$ 0.07
15. min	5.16 $\pm$ 0.74	0.14 $\pm$ 0.05	7.98 $\pm$ 0.84	0.27 $\pm$ 0.06
20. min	5.00 $\pm$ 0.52	0.13 $\pm$ 0.05	7.30 $\pm$ 0.95	0.26 $\pm$ 0.06

Values are mean $\pm$ SEM.

groups on the basis of SL curves. In the first group ($n = 5$) subjects had a lactate peak at 1 min and the second peak at 8 min after the test. In the second group ($n = 3$) each lactate peak occurred 3 min later. Due to the low number of samples we did not find significant differences between the two groups of athletes concerning our measured parameters. In controls without any regular changes in SL level, we found high individual variability in lactate profile (Table 2). BL level was the highest at one min after the exercise (10.7 ± 0.62 (SEM) mM/l) then it decreased gradually in both groups (Table 2). BL level is elevated in every measurement similarly to SL level (Fig. 2). The profile of lactate concentration curves in the two fluids is very similar in athletes and it differs from the controls.

In addition, we found significant correlation between BL and SL in athletes ($r = 0.511$; $p < 0.001$) and non-athletes, too ($r = 0.385$; $p < 0.001$) after maximum

Table 3
Significant differences between the test groups

Variables	Athletes	Controls
Training volume (h/week)	15.9±2.78**	1.69±0.87**
Test time (min)	19.14±1.31**	9.21±1.44**
Body fat (%)	9.36±1.93*	19.31±3.99*
Fat mass (kg)	5.58±1.01*	13.53±3.22*
Total body water (%)	64.75±1.56*	56.63±2.62*
VLF (ms ²)	3342.61±1152.26*	124.42±31.78*
VLF%	59.06±4.01*	76.97±2.86*
LF (ms ²)	1393.72±316.25*	35.73±10.16*
LF%	30.53±3.60*	20.36±2.24*
HF (ms ²)	575.31±240.90*	4.72±2.05*
HF%	10.39±1.62*	2.68±0.77*
LF/HF ratio	334.15±45.53**	951.09±120.48**
Number of heart beats	913.50±86.97**	447.75±12.97**
Mean RR (ms)	347.00±29.09**	673.88±18.88**
HRmin (during test, bpm)	67.63±4.88*	89.88±6.93*
HR (1 min after test, bpm)	132.13±4.14*	146.00±5.11*

Values are mean ±SEM; * $p < 0.05$; ** $p < 0.01$.

intensity, but correlation is lower in controls. As it was expected lactate concentration was higher in controls at each time point of the measurement.

Differences between groups were revealed as presented in Table 3 showing significant differences in the parameters of body composition (fat mass, body fat, and whole body water), circulatory parameters (maximal HR, recovery HR) and changes in sympathetic-parasympathetic balance (RR variability) in test groups.

Pearson correlation analyses between 0, 12, 15, 20 min SL level and total body water showed significant, negative correlation ($r = -0.547$; $r = -0.688$; $r = -0.683$; $r = -0.677$; $p < 0.05$), meaning that dehydration decreases the salivary flow rate and the secretion becomes slower by time.

Maximal HR during exercise was correlated significantly with maximal SL concentration ($r = 0.506$; $p < 0.05$). Strong correlation was observed between 4, 8, 12, 15, 20 min BL, 4, 12 min SL concentration and HR measured one min after the test ($r = 0.620$; $r = 0.740$; $r = 0.599$; $r = 0.709$; $r = 0.559$; $r = 0.562$; $r = 0.531$; $p < 0.05$).

We noted strong relationship between the 4, 8, 12, 20 min lactate concentrations in saliva and the mean RR, measured during the exercise ($r = 0.537$; $r = 0.544$; $r = 0.557$; $r = 0.510$; $p < 0.05$).

Furthermore, significant correlations were found 4, 8, 12, 20 min lactate concentration in saliva and NHB during the test ($r = -0.538$; $r = -0.532$; $r = -0.516$; $r = -0.513$; $p < 0.05$).

Since SL measurements in athletes after the exercise test revealed to groups, we analyzed their HR data in two groups as well. Due to the low number of subjects in each groups only tendencies can be presented. Comparing HR data obtained from the exercise test between athlete group 1 (G1, SL peaks at 1 and 8 min) and group 2 (G2, SL peaks at 4 and 12 min) the following results were seen. Before the test resting mean RR was 376.8 ± 35.1 ms in G1 and 297.33 ± 42.6 ms in G2. In the last 5 min of the treadmill exercise test mean RR was 319.0 ± 4.93 ms in G1 and 319.0 ± 5.56 ms in G2. After the test recovery HR data were recorded for further 5 min, and in the last min of this period mean RR was 519.0 ± 16.33 ms (G1) and 539.66 ± 3.48 ms (G2), respectively. Differences are recognizable in the LF parameters between G1 and G2 (in rest 1112.82 ± 397.36 ms², 1861.86 ± 481.39 ms²; in exercise 1.04 ± 0.79 , 0.25 ± 0.07 ms²; in recovery 43.55 ± 13.04 ms² and 119.47 ± 45.35 ms²), respectively. In this parameter the differences are almost significant ($p < 0.069$). LF/HF ratio was also calculated in each stages of the study. At rest (before the test) 365.7 ± 53.68 ms² in G1 and 281.56 ± 86.76 ms² in G2, while in the last 5 min of the test 139.13 ± 27.24 ms² (G1) and 131.56 ± 24.13 ms² (G2). The LF/HF ratio in recovery was calculated as 944.86 ± 195.87 ms² (G1) and 1911.03 ± 564.6 ms² (G2).

DISCUSSION

In our study we have found differences in SL responses in athletes and non-athletes after maximal exercise, while the changes in BL concentration were similar in both groups. Stronger correlation was noted between SL and BL in athletes ($r = 0.511$; $p < 0.001$), than in controls ($r = 0.385$; $p < 0.001$). Before this investigation our team conducted a study in which we got somewhat different results than other researchers [27]. In spite of other authors we have found two SL peaks post-exercise, instead of one, but we were not able to explain this result fully.

In body composition we did not register important differences in test groups (except body fat), which would explain changes of SL level.

Physiological differences between athletes and non-athletes can cause different SL responses in both groups. According to our study, these can be defined primarily as three physiological changes: adaptation of the nervous system and circulatory system to exercise, and altered hydration status. Although the two athlete groups do not differ in the maximal HR and the time spent on the treadmill, data obtained from HR analyses in our study show that RR is smaller in the second athlete group in rest already, and at the end of the recovery period, too. The higher sympathetic tone is clear concerning LF and LF/HF ratio. LF parameters are higher in the second group

at rest and at recovery, too. The differences disappear at maximal exercise. Concerning LH/HF ratio it is much higher at recovery in the second group indicating that indeed high sympathetic tone slows saliva secretion and lactate appearance in saliva.

Unlike in non-athletes regular physical training resulted in an increase in parasympathetic activity and decrease of sympathetic cardiac modulation [8] as it was observed in our groups. The parasympathetic impulses evoke secretion of salivary fluid whilst the activation of sympathetic nerves will cause vasoconstriction in vessels of salivary glands, consequently less saliva is produced [20]. HR data between athletes and controls in accordance with the test time show the low vegetative functions in control, the low vagal tone resulting in instable metabolism and saliva secretion.

The effect of autonomic nervous system can be detected in changes of cardiac function. The physical fitness may be estimated with recovery of heart rate after exercise so it can potentially measure the training status [9].

Saliva reflects qualitative and quantitative changes of plasma components in numerous aspects [25]. It is also known that there is a close relationship between the body hydration status and saliva flow rate. Dehydration can be associated with the decrease of salivation [28]. Our results show that there is a time delay between BL level and SL and it can be different in endurance athletes and non-athletes. Santos et al. [23] considered that lactate filtrates from the blood to salivary gland in a certain time, however it is not clear yet how long it might take and which parameters influence it. Since HR changes are faster after exercise in trained persons, showing a stronger parasympathetic nerve tone, salivary flow will return to the normal rate faster in trained athletes.

Nowadays it is still an important question, whether only exercise will induce SL alteration or this is influenced by permeability of blood-saliva barrier, salivary viscosity and flow rate, too [11, 26].

Our data suggest that measuring SL might be a suitable method to test the efficiency of training and the current status of vegetative functions. It is obvious that measuring the effect of training we all have better potential with non-invasive saliva tests of athletes from invasive blood tests. Further experiments are needed to determine how BL and SL levels are influenced by physiological factors.

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