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Original Article

Convergent validity of a simplified device and relationship between blood lactate and salivary lactate after a vertical squat jump in healthy non-athletes

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Abstract. [Purpose] The aims of this study were 1) to examine the convergent validity between Lactate pro 2 and a standard JCA-BM 8000 automatic analyzer using salivary lactate and 2) to investigate the relationship between blood and salivary lactate levels after a vertical squat jump. [Participants and Methods] Healthy non-athletes participated in this observational study. The participants performed a vertical squat jump for 1 min 30 s. Blood and salivary lactate levels were measured before and after exercise using Lactate Pro 2. [Results] The intraclass correlation coefficient between Lactate Pro 2 and the JCA-BM 8000 automatic analyzer was 0.773, which can be considered as substantial convergent validity. However, in some samples, the salivary lactate level was out of the measurable range, and numerical values could not be obtained. The cross-correlation function between the blood and salivary lactate levels was 0.535 at lag 0 and 0.750 at lag 1, which indicated a 5-min lag between the salivary and blood lactate values. [Conclusion] Salivary lactate levels can be easily measured using Lactate Pro 2, although its sensitivity needs to be resolved. Further research is required for salivary lactate level, which can be collected non-invasively, to be used as an alternative parameter to blood lactate level.
Key words: Blood lactate, Salivary lactate, Vertical squat jump

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INTRODUCTION

In the field of sports rehabilitation and physical therapy, it is important to comprehensively evaluate the responses to exercise so as to accurately prescribe and assess the effectiveness of the exercise program. These responses to exercise have been highly documented and include the morphological[1], neurological[1)], biochemical[2)], biomechanical[3)], metabolic[4)], cardiovascular[5–7)], respiratory[8, 9)], cognitive[10, 11)], and emotional[12, 13)] adaptations to exercise.

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A common method of exercise prescription, especially in the area of cardiac rehabilitation, is based on the anaerobic threshold (AT)[14] calculated from the cardiopulmonary exercise testing (CPX). CPX requires large-scale equipment and the number of facilities that can implement CPX is limited by this requirement. On the other hand, blood lactate (BLA) concentration and the lactate threshold (LT) which is the point of exponential rise in BLA during a graded incremental exercise test, is often used in the field of sports science[15–17]. Lactate is a metabolic marker that provides information on aerobic capacity[18], and LT concepts (both BLA measurements and gas exchange measurements) have been shown to be highly correlated with endurance performance[19]. Therefore, it has been proposed that both the LT and AT may signal the aerobic-anaerobic transition, and they may be used for exercise prescription, as well as for performance evaluation in endurance sports[20–22]. Casaburi et al. reported that high-intensity training, compared with low intensity training, clearly reduced BLA during exercise and consequently improved ventilation, dyspnea, and exercise endurance[23]. One of the main mechanisms of increased exercise endurance is the improvement in skeletal muscle function. Specifically, Hambrecht et al. found enhanced oxidative enzyme activity in the working skeletal muscles and a concomitant shift from type II to type I fibers which contains more oxidase compared to type II fast twitch fibers in patients with chronic heart failure[24]. Moreover, decrease in lactate concentration at the same exercise intensity has been reported to correlate with increased mitochondrial density and oxidase activity[24].

Therefore, BLA measurements as a metabolic index for the assessment of the aerobic capacity can be a useful evaluation technique for subjects, regardless of the presence or absence of disease. However, BLA measurements by physical therapists are not being widely performed because most of the existing methods are more or less invasive, requiring blood collection from the fingertips[25] or earlobes[26]. In contrast, the use of salivary lactate (SLA) which can be collected non-invasively, compared with BLA, has greater potential for use by physical therapists since SLA has been shown to have a high positive correlation with BLA. Segura et al. used the YSI 1500 model (Yellow Spring Instruments, Ohio, USA), for both BLA and SLA measurements and reported that although the SLA concentration was about 15% compared with BLA, the pattern of evolution during the exercise test was similar[27]. They also reported that BLA and SLA were positively correlated (r=0.81) during the performance of a maximum graded exercise test on a cycle ergometer[27]. Similarly, Santos et al. measured BLA and SLA at 0 km, and every 6 km thereafter during a 30-km run using the same device with Segura et al. Their results showed that there was a strong correlation between absolute BLA and SLA (r=0.77)[18]. Tekas et al. reported a positive correlation between BLA and SLA (r=0.511) before and after the maximum intensity Astrand treadmill test. They used a portable lactate analyzer, Lactate Scout (SensLab, Leipzig, Germany) for BLA measurement, and SLA was measured by spectrophotometric analysis (a method to measure the amount of Reduced Nicotinamide Adenine Dinucleotide or NADH that accompanies pyruvate when lactate is oxidized in the presence of Nicotinamide Adenine Dinucleotide or NAD^+)[28]. Although a positive correlation between BLA and SLA has been reported in cycling and running tasks, there is no gold standard device for measuring SLA, and the absolute value of SLA measured by each device has been shown to have a considerable range.

Lactate Pro 2 (Arkray, Kyoto, Japan) is a point-of-care device for measuring BLA using whole blood. It is an updated model of Lactate Pro[29, 30], and has been used in the sports and medical fields because it is portable and the results can be obtained in 15 sec[31–33]. Lactate Pro was reported to have high convergent validity with other lactate analyzers (ABL 700 Series Acid-Base analyser[34], YSI 2300 Stat lactate analyser[34], Accusport Lactate Meter[34] and YSI 1500[35]). In addition, a positive correlation (r=0.99) was reported between Lactate Pro and Lactate Pro 2 when the same blood sample was used to measure BLA[34]. However, whether Lactate Pro 2 could be used to measure SLA is unknown and have yet to be investigated.

Furthermore, while cycling and long-distance running are good examples of anaerobic exercises, they may not be convenient for assessing AT as they are time-consuming as well as dependent on equipment. On the other hand, a vertical squat jump (VSJ) is a form of short-duration, high-intensity anaerobic activity that has been used to assess AT in many sports[36]. It is also widely used in rehabilitation of athletes in order to return them to competition[37]. VSJ is a simple task that does not require special equipment and has low complexity[38].

Therefore, the first aim of this study was to examine the convergent validity between Lactate pro 2 and a standard JCA-BM 8000 automatic analyzer (Japan Electron Optics Laboratories, Tokyo, Japan), using SLA as the measurement variable. The second aim of this study was to investigate the relationship between BLA and SLA measured by Lactate Pro 2 after a short-duration, high-intensity anaerobic activity such as a VSJ.

PARTICIPANTS AND METHODS

This study was conducted in two parts, corresponding to the two stated aims. The first part, which investigated the convergent validity of the Lactate Pro 2 device with a standard JCA-BM 8000 automatic analyzer was conducted 2 months before the main study (Lactate Pro 2 device test). Lactate Pro 2 is a commercially available simplified device for BLA measurement with a measurable range of 0.5–25.0 mmol/L; values below 0.5 mmol/L and beyond 25.0 mmol/L are displayed as “Lo” and “Hi,” respectively. Five participants (2 females, 3 males) performed the VSJ exercise, and only samples of saliva were collected before VSJ (pre), immediately after VSJ (post 0), 3 min after VSJ (post 3), and thereafter at every 2-min interval until 30 min (post 5, post 7, …, post 29). After measuring the lactate concentration using the Lactate Pro 2, the same sample was centrifuged at 6,000 rpm for 5 min at 4 °C. The supernatants were then mixed with 0.8 N perchloric acid solution for enzymatic concentration measurement using lactate oxidase.

The second part of the study was to investigate the relationship between BLA and SLA measured by Lactate Pro 2 after a VSJ (VSJ test). Twenty healthy non-athletic males participated in this study. All participants were informed of the purpose of

this study including the risks involved, and their written consent was obtained. They were instructed to refrain from intense exercise a day before the trial. On the day of the trial, the participants had to abstain from eating and drinking, with the exception of water, from 2 h before the commencement of the trials. All measurements were conducted in a well-ventilated room with a temperature of 26.1 ± 0.6 °C and humidity of 51.2 ± 6.5%.

Prior to the trials, the procedure for performing the VSJ was demonstrated and explained to each participant. VSJ was explained as follows: 1) In the squat phase, the feet are spread shoulder width apart, with knee flexion at 90° and trunk and lower legs parallel to each other. 2) In the jump phase, the jump should be as high as possible while swinging the upper limbs. The participants were required to jump at a tempo of 46 jumps per minute and to continue the task for 1 min and 30 s. To ensure the maximum effort from the participants, they were not notified of the exercise time in advance; instead, they were instructed to continue the exercise until the examiner signaled for them to stop. During the recovery phase, they rested in a sitting position for 30 min.

Both BLA and SLA were measured using the Lactate Pro 2 before exercise (pre), immediately after exercise (post 0), and every 5 min thereafter (post 5, post 10, post 15, until post 30) during the recovery phase. BLA samples were collected from a small amount of blood taken from the fingertip using a medical puncturing device (Naturalette Petit, Arkray), while SLA samples were collected from saliva which was directly expectorated onto a clean plate. The participants were instructed to brush their teeth during the rest phase (before exercise) and rinse their mouths with distilled water after exercise to remove oral debris and to clean oral surfaces[28, 39]. A Lactate Pro2 sensor was directly attached to each sample for the measurement of BLA and SLA concentrations. The interval between the BLA and SLA measurements was kept within 10 s.

Data analysis was also conducted in two parts, corresponding to the two stated aims. In the first analysis, the convergent validity between concentrations of SLA measured using Lactate Pro2 and those using the JCA-BM 8000 automatic analyzer were assessed by intraclass correlation coefficient (ICC) with a two-way random model (absolute agreement) to determine the degree of agreement. Convergent validity was judged to be moderate at ICC 0.41–0.60, substantial at ICC 0.61–0.80, and almost perfect above ICC 0.80[40]. Before the analysis, the data were normalized by dividing it with the baseline (pre) value in order to compare the different absolute values. In the measurement by Lactate Pro 2, 0.4 mmol/L or less is displayed as “Lo”. Since it cannot be calculated, it was treated as a missing value in this study. In the second analysis, the relationship between BLA and SLA was analyzed using ICC and cross correlation analysis between normalized BLA and SLA. Cross correlation is a correlation obtained when the data is shifted along the time axis in two time series data, and therefore the relationship of deviation (time lag) can be investigated. The cross correlation function (CCF) is a correlation coefficient calculated at different time lags. All values were expressed as mean ± standard deviation (SD). All statistical analyses were performed using PASW Statistics software (version 26.0, SPSS, Inc., Chicago, IL, USA). All experimental protocols in this study were reviewed and approved by the Medical Ethics Committee of Shinshu University School of Medicine (Approval No. 4120).

RESULTS

For the convergent validity of Lactate Pro 2 device test, the characteristics of the 5 participants are shown in Table 1. Sixteen samples were collected from each of the 5 participants. Seven samples out of 80 were displayed as “Lo”, and these were treated as missing values during the analyses. The SLA values and normalized SLA values measured by Lactate Pro 2 and an automatic analyzer are shown in Table 2. SLA values were normalized by dividing them with the baseline pre values. One participant had a baseline pre value of “Lo” and since normalization could not be carried out, she was excluded from further analysis. The ICCs between Lactate Pro 2 and an automatic analyzer for each subject are shown in Table 3. The ICCs ranged from 0.568 to 0.763 (overall ICC was 0.773, 95% confidence interval: 0.678 to 0.844).

In VSJ test, none of the participants dropped out and the data for all 20 were analyzed. The participants’ characteristics in VSJ test are shown in Table 1. Eight samples of BLA and SLA were collected from each of the 20 participants. “Lo” was displayed in 20 out of 160 samples from SLA, and 5 participants who displayed “Lo” in the pre measurement were excluded from the analysis. The BLA and SLA concentrations before and after VSJ, and ICCs are shown in Table 4. Compared with SLA, BLA generally peaked earlier in 14 out of the 15 participants. Therefore, a cross correlation analysis was performed with the results shown in Table 5. The Cross Correlation Function (CCF) at lag 0 and lag 1 were 0.535 and 0.750 respectively, indicating that SLA lagged by one time period (i.e. 5 min) behind BLA.

Table 1. Participants’ characteristics of Lactate Pro 2 device test and VSJ test

	Lactate pro 2 device test		VSJ test
	Female (n=2)	Male (n=3)	Male (n=20)
Age (years)	20.5 ± 0.5	20.3 ± 0.5	20.2 ± 1.3
Height (m)	1.5 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
Body weight (kg)	49.0 ± 2.0	70.9 ± 3.8	61.9 ± 7.5
BMI (kg/m^2)	20.8 ± 0.9	21.6 ± 1.4	20.8 ± 2.1

VSJ: vertical squat jump; BMI: body mass index. Data are mean ± SD.

Table 2. SLA values and normalized SLA values measured by Lactate Pro 2 and JCA-BM 8000 automatic analyzer (pre values used as baseline for normalization) (n=4)

	Lactate Pro 2 (mmol/L)	Automatic analyzer (mmol/L)	Normalized Lactate Pro 2	Normalized automatic analyzer
Pre	0.63 ± 0.13	0.22 ± 0.10	1.00 ± 0.00	1.00 ± 0.00
Post 0	0.70 ± 0.00	0.14 ± 0.05	1.40 ± 0.00	0.69 ± 0.24
Post 3	0.73 ± 0.26	0.23 ± 0.04	1.08 ± 0.22	1.21 ± 0.45
Post 5	1.00 ± 0.37	0.27 ± 0.09	1.64 ± 0.71	1.35 ± 0.52
Post 7	1.53 ± 0.75	0.48 ± 0.16	2.46 ± 1.22	2.57 ± 1.27
Post 9	2.15 ± 0.84	0.72 ± 0.34	3.51 ± 1.55	3.91 ± 2.64
Post 11	2.75 ± 0.61	0.79 ± 0.33	4.70 ± 1.72	4.13 ± 2.45
Post 13	1.70 ± 0.21	0.57 ± 0.29	2.85 ± 0.74	3.25 ± 2.40
Post 15	2.18 ± 0.62	0.62 ± 0.18	3.72 ± 1.57	3.45 ± 1.99
Post 17	2.35 ± 0.74	0.68 ± 0.25	4.13 ± 1.97	3.35 ± 1.03
Post 19	1.73 ± 0.63	0.58 ± 0.20	2.83 ± 1.13	3.00 ± 1.64
Post 21	1.80 ± 0.49	0.60 ± 0.14	3.15 ± 1.40	3.04 ± 0.95
Post 23	1.73 ± 0.54	0.64 ± 0.24	3.05 ± 1.47	3.32 ± 1.77
Post 25	1.20 ± 0.37	0.35 ± 0.13	2.09 ± 0.95	1.74 ± 0.70
Post 27	1.15 ± 0.23	0.37 ± 0.10	1.94 ± 0.62	1.97 ± 0.93
Post 29	1.40 ± 0.22	0.37 ± 0.16	2.41 ± 0.82	1.97 ± 1.29

SLA: salivary lactate. Data are mean ± SD.

Table 3. The convergent validity of normalized SLA values between Lactate Pro 2 and JCA-BM 8000 automatic analyzer

Participant	ICC (2, 1)	95% CI	
		Lower	Upper
A	0.634	−0.005	0.864
B	0.568	−0.031	0.830
C	0.763	0.498	0.895
D	0.552	0.089	0.798
Total (n=4)	0.773	0.678	0.844

SLA: salivary lactate; ICC: intraclass correlation coefficients; CI: confidence interval.

Table 4. BLA and SLA concentrations before and after VSJ (n=15)

	BLA (mmol/L)	SLA (mmol/L)	Normalized BLA	Normalized SLA	ICC (2, 1)	95% CI	
						Lower	Upper
Pre	1.67 ± 0.38	0.72 ± 0.22	1.00 ± 0.00	1.00 ± 0.00	—	—	
Post 0	10.39 ± 2.50	0.71 ± 0.34	6.63 ± 2.24	1.05 ± 0.60	0.012	-0.035	0.153
Post 5	13.31 ± 1.32	1.41 ± 0.87	8.38 ± 2.07	2.13 ± 1.59	−0.039	−0.107	0.159
Post 10	12.02 ± 1.42	2.13 ± 1.32	7.57 ± 2.02	3.25 ± 2.37	−0.004	−0.143	0.262
Post 15	9.82 ± 1.60	2.20 ± 1.25	6.19 ± 1.84	3.19 ± 1.86	−0.061	−0.252	0.273
Post 20	8.95 ± 1.68	2.14 ± 1.36	5.57 ± 1.45	2.93 ± 1.65	0.005	−0.166	0.308
Post 25	7.09 ± 1.29	1.34 ± 0.58	4.37 ± 1.26	2.02 ± 1.06	−0.139	−0.279	0.270
Post 30	5.95 ± 0.75	1.14 ± 0.60	3.15 ± 0.57	1.69 ± 0.91	−0.646	−0.685	0.035

BLA: blood lactate; SLA: salivary lactate; VSJ: vertical squat jump; ICC: intraclass correlation coefficients; CI: confidence interval. Data are mean ± SD.

Table 5. Cross correlation analysis of two time series data of normalized BLA and SLA

Lag	Cross correlation	Standard error
−6	0.235	0.707
−5	0.059	0.577
−4	−0.235	0.500
−3	−0.448	0.447
−2	−0.554	0.408
−1	−0.275	0.378
0	0.535	0.354
1	0.750	0.378
2	0.400	0.408
3	−0.118	0.447
4	−0.324	0.500
5	−0.325	0.577
6	0.000	0.707

DISCUSSION

One participant (female) out of 5 (2 females, 3 males) in the Lactate Pro 2 device test, and 5 participants out of 20 (all males) in VSJ test were excluded from the analysis because of the "Lo" display in the pre (baseline) measurement of SLA. This is due to the sensitivity of Lactate Pro 2, which is the limitation of a simplified device with a measurable range of 0.5–25.0 mmol/L. In contrast, BLA measurements using Lactate Pro 2 were recorded for all participants without any problems, and this was also similarly reported by others in the sports[31] and medical fields[32, 33]. However, many studies have shown that the absolute values SLA were lower than BLA in general[18, 27, 28, 41]. The inability to measure small values may be the limitation of measuring SLA with Lactate Pro 2.

Lactate Pro 2 is portable, requires less sample volume (0.3 µL) than other devices, and takes less time (15 s) to obtain the results. Moreover, it is easy for patients and/or caregivers to measure because there is no need for pipetting to adjust the sample volume. Our results demonstrated that the ICCs between Lactate Pro 2 and JCA-BM 8000 automatic analyzer was 0.773, which can be considered as having substantial convergent validity and has the potential to be used in clinical practice, at least within the measurable range. However, there was a difference in the absolute values of SLA measured by the two devices, and we used normalized values for data analysis. It may be necessary to establish a standardized method for SLA measurements with the Lactate Pro 2 in order to be able to generalize SLA results with other similar devices.

In VSJ test, we investigated the relationship between BLA and SLA after a VSJ task as a short-term, high-intensity anaerobic exercise. Our results showed that the peak BLA concentration was 13.39 ± 1.23 mmol/L. This was similar to the results by Tekus et al. who reported that peak BLA was 11.20 ± 0.63 mmol/L after a maximal treadmill test in non-athletes[28]. This suggests that while VSJ and maximal treadmill test may be considered to be similar tasks, VSJ may be more convenient as it does not require special equipment and can be completed in a shorter time. In contrast, Santos et al. reported that peak BLA concentration was 3.15 ± 0.42 mmol/L in participants during a 30-km race[18]. This difference may be partially due to the different type of activity used in their study. Lactate is mainly produced in type II fibers (fast fibers)[42]. In VSJ, which requires instantaneous force, the fast muscles acted as agonist muscles and may result in an increase in BLA compared to long-distance running.

Our results also showed that there was no high positive correlation between the normalized BLA and SLA at each time point in the ICCs. There have been no reports in previous studies showing the concurrent validity of BLA and SLA at certain points in time. Most papers have evaluated BLA and SLA movements before and after exercise using Pearson's correlation. However, this data analysis method may not be appropriate for investigating the within-subject relationship between BLA and SLA. In this study, we applied a cross correlation analysis to compare the relationship of BLA and SLA after VSJ because they can be considered as time series data. Our results showed that SLA lagged by 5 min behind BLA (CCF=0.750 at lag 1). However, Santos et al. reported that BLA and SLA changed in tandem without time lag[18]. Segura et al. applied a gradual load exercise using an ergometer as the exercise task, and also showed that BLA and SLA changed in tandem with no time lag[27]. By contrast, following 3,000-m and 400-m run tests, Ohkuwa et al. reported that the SLA peaked 5 to 10 min after BLA[40], similar to our results. One of the possible reasons for this time lag may be the difference in exercise tasks. Goodwin et al. mentioned that BLA peaked 3 to 8 min after exercise task that lasts 30 to 120 s was performed with maximum effort, with BLA gradually increasing at first and rapidly towards the end of the task during a gradual load exercise[43]. The origin of SLA is mainly from BLA produced by exercise, so that if the increase of BLA is physiologically different due to exercise

tasks, the subsequent release to saliva may also be different. The composition of saliva is affected by different salivary glands and there are wide individual variations[44]. Although it is difficult to track SLA completely, further research is required to clarify this time lag.

In conclusion, our study demonstrated there was a possibility that SLA can be easily measured in clinical situations using Lactate Pro 2, although the sensitivity ("Lo" display) may be a cause for concern. Our study also demonstrated that BLA after short-term, high-intensity VSJ exercise did not change in tandem with SLA, and there was a time-lag difference of 5 min. Further research is required before SLA can be considered as an alternative to BLA for the assessment of lactate levels.

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Conflict of interest

None.

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