

The Influence of Sampling Site on Blood Lactate Response to Steady State Exercise

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Abstract: In sports sciences, blood lactate is commonly measured for performance testing or setting exercise intensity. Differences in measuring lactate concentration over time and across sampling sites during constant exercise are unknown. We aimed to compare blood lactate concentration across sampling sites during constant exercise. Eight participants performed three bouts of 20-min steady-state cycle exercise, consisting of 45, 60, and 75% of peak oxygen uptake ($\dot{V}O_{2peak}$). Blood lactate levels were measured simultaneously from the fingertip and earlobe every 5 min during exercise. The time course analysis revealed that lactate level in the fingertip was significantly higher than in the earlobe at either work intensity ($P < 0.05$). A significant interaction effect (site \times time) was observed at 60% $\dot{V}O_{2peak}$ ($P < 0.001$), which indicated that the difference in samples across sampling sites decreased over time. The levels in the fingertip samples were higher at 5 min ($P < 0.05$); this difference was not noted after 10 min. Similarly, the fingertip values were significantly higher until 10 min ($P < 0.05$), but not after 15 min at 75% $\dot{V}O_{2peak}$. These findings suggest that the sampling site may affect the time course of change in lactate value under a constant workload.

Key words: Lactic acid, constant work, cycle ergometry, lactate steady state.

1. Introduction

Blood lactate concentrations are often measured during exercise prescription and performance testing of athletes [1-2]. The development of portable equipment that needs less amount of blood sample for measuring blood lactate concentration was feasible under laboratory and field conditions. Consequently, the measurement of blood lactate concentration expanded to various fields of sports for the enhancement of athlete performance and health benefits.

The blood sampling sites during exercise mostly include the fingertip, earlobe, and toe [3]. Several studies have examined whether blood lactate level differ across sampling sites during and after exercise [3-7]. As no significant difference across sampling

sites was observed [3], exercise physiologists have been suggested to use lactate interchangeably for physiological assessment [7]. Conversely, several studies have documented a difference in blood lactate values across sampling sites during and after exercise [4-5]. These data showed that measurements in the fingertip consistently indicate higher blood lactate levels compared with earlobe measurements. Several factors can influence the sampling site difference in blood lactate concentration between the fingertip and the earlobe. For instance, sweat, which contains high lactate levels [8-9], might be first detected in the palm before being detectable in the ear [10]. Furthermore, sympathetic vasoconstriction, which reduces microcirculation, followed by the enhancement of glycolytic metabolism and lactate production [11], is more active in the finger site than in other sites [12]. These data suggest that the fingertip is more affected than the earlobe by such physiological responses.

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Furthermore, prolonged exercise might also affect the changes in blood lactate concentration among sampling sites because of increased sweating and attenuation of the regional vasoconstrictor after the increment in internal temperature with exercise progression [13]. However, to our knowledge, previous studies concerning sampling site differences often used a short duration of incremental exercise [14-15] and were not focused on constant exercise.

In the field of sports science, lactate threshold and onset of blood lactate accumulation are mostly used in various exercise fields for a long time. By contrast, blood lactate level is used not only as an incremental test but also during continuous exercise. In the recent decade, MLSS (maximal lactate steady state), defined as the highest concentration of blood lactate that can be maintained during prolonged steady-state exercise, was indicated as a superior measurement of physical performance as compared with the blood lactate threshold during incremental exercise tests [16]. The single-visit methods for detecting MLSS have been studied in the laboratory [17-18] and fields [19] because MLSS measurement is needed for several repeated tests on separate days. However, information about the surrogate method for the analysis of MLSS in a single visit remains scarce. By focusing on sampling sites in these studies, different sampling sites were used, including the earlobe [17, 19] and fingertips [18]. If the time-course variation of blood lactate level during constant exercise is different across sampling sites, the changes in blood lactate concentration do not accurately indicate the balance between lactate appearance and disappearance. Information about the blood lactate difference among sampling sites during continuous exercise is needed for the future development of MLSS study.

The aim of this study was to compare the effect of sampling sites on blood lactate concentration during steady-state exercise. We hypothesized that the difference across sampling sites would be small over time during exercise, as regional lactate production

after vasoconstriction would be more active in the fingertip than in the earlobe at the beginning of exercise [5, 12, 20].

2. Methods

2.1 Participants

Eight men recreational athletes competing in track and field, rugby, and baseball volunteered to participate in the study. The mean (SE) age, height, mass and peak oxygen uptake ($\dot{V}O_{2peak}$) were 23 (1) years, 171.2 (1.8) cm, 66.3 (2.4) kg and 49.8 (3.6) ml·kg⁻¹·min⁻¹, respectively. All procedures were approved by institutional review boards, which conformed to the Helsinki Declaration. All subjects provided written informed consent before participating.

2.2 Experimental Design & Procedures

Each subject visited the laboratory on four separate occasions. On the first testing day, the subjects underwent a progressive exercise test. During the remaining three days, participants completed three continuous steady state 20-min cycle exercises (in random order). Tests took place at the same time of day, more than 48 h were set among tests, 24 h apart only after 45% $\dot{V}O_{2peak}$. Subjects were asked to refrain from vigorous exercise and caffeine intake the day before testing. Food intake was avoided 2 h before testing.

2.3 Preliminary Testing

For measurement of $\dot{V}O_{2peak}$, the incremental exercise test was conducted on a cycle ergometer (AEROBIKE 75XL, COMBI, Japan). The initial workload commenced at 90 watts for 4 min followed by increases in power of 30 watts every 2 min until volitional exhaustion. Subjects were instructed to maintain a pedal cadence 60 rpm throughout the exercise. Respiratory gas was analyzed using the Douglas bag method. Concentrations of CO₂ and O₂ were determined using a mass spectrometer (WSMR-1400, WESTRON, Japan). The volume of expired air was determined using a certified dry gas

volume meter (DC-5, Shinagawa Seisakusho, Japan). The highest value of oxygen uptake obtained throughout the exercise protocol was used as the $\dot{V}O_{2peak}$. The intensities of constant work load corresponding to 45, 60, and 75% $\dot{V}O_{2peak}$ were calculated using data from the incremental test.

2.4 Experimental Protocol

After arriving to the laboratory, participants remained at rest on a chair for more than 30 min. Subsequently, participants sat on a cycle ergometer for 5 min. During rest, blood lactate concentrations were measured in capillary blood samples collected simultaneously from the earlobe and fingertip. While warming up, 20% of $\dot{V}O_2$ reserve of 5 min was approved, followed by steady state exercise for 20 min. Blood samples were measured from two sample sites every 5 min during steady state exercise.

2.5 Blood Sampling & Preparations

Sample sites were cleaned and dried with a swab before sampling. Blood lactate concentrations were measured in capillary blood samples collected from simultaneously (less than 30 sec) earlobe and fingertip using two devices by the same examiner. A 5 μ l blood samples were taken at rest and every 5 min during exercise, while the participant cycled continually. Missing values because of the collection of an insufficient volume of blood sampling to detect, which included two fingertip samples and five earlobe samples, were interpolated by quadratic least squares regression fit that consisted of the other three samples obtained during exercise. If more than two data points were missing, the subject was excluded. In total, seven subjects were measured at 45% and 75% of $\dot{V}O_{2peak}$ exercise condition.

The blood samples were determined by portable lactate analyzer (Lactate Pro, Arkray, Japan), which has been shown to have an acceptable level of precision and accuracy [21]. The minimum range of this equipment was $0.8 \text{ mmol} \cdot \text{L}^{-1}$, if this tool showed a level

below detection, it was assumed to be $0.7 \text{ mmol} \cdot \text{L}^{-1}$.

2.6 Statistical Analysis

The correlation between fingertip and earlobe was analyzed using Pearson's correlation coefficient. Bland-Altman plot for agreement between sample sites at each time were evaluated [22], and 95% CI (confidence intervals) were calculated (95% CI = 1.96 s). Pearson's correlation coefficient was also subsequently employed to identify possible adjustments for comparison among the sample sites.

Comparisons of measures between the fingertip and earlobe blood lactate concentrations during exercise were evaluated by a two-way ANOVA (analysis of variance) with repeated measures. Holm's post hoc test was used to determine the differences in sampling site at each time and time effect after 5 min. Paired *t*-tests assessed the validity of differences across sampling sites at rest. The lactate MD (mean difference) across sampling sites and 95% confidence intervals of difference (CI) were calculated. Effect sizes were calculated using partial eta square (ηp^2) values (ANOVA) and Cohen's effect size (*d*) (*t* test). Significance was assumed when $P < 0.05$ throughout the investigation.

3. Results

A significant relationship between blood lactate concentration of the fingertip and the earlobe was observed for all comparable data ($r = 0.975$, CI 0.96-0.98, $P < 0.001$; Fig. 1).

Table 1 and Fig. 2 illustrate the capillary blood lactate concentration for the two sites at rest and during constant exercise.

There were no significant differences between fingertip and earlobe at rest under 45% (MD 0.11 $\text{mmol} \cdot \text{L}^{-1}$, CI -0.32-0.55, $P = 0.547$, $d = 0.24$) and 75% $\dot{V}O_{2peak}$ (MD 0.03 $\text{mmol} \cdot \text{L}^{-1}$, CI -0.14-0.19, $P = 0.689$, $d = 0.16$), though the fingertip value was higher than that of the earlobe at 60% $\dot{V}O_{2peak}$ (MD 0.23 $\text{mmol} \cdot \text{L}^{-1}$, CI 0.09-0.36, $P = 0.005$, $d = 1.42$).

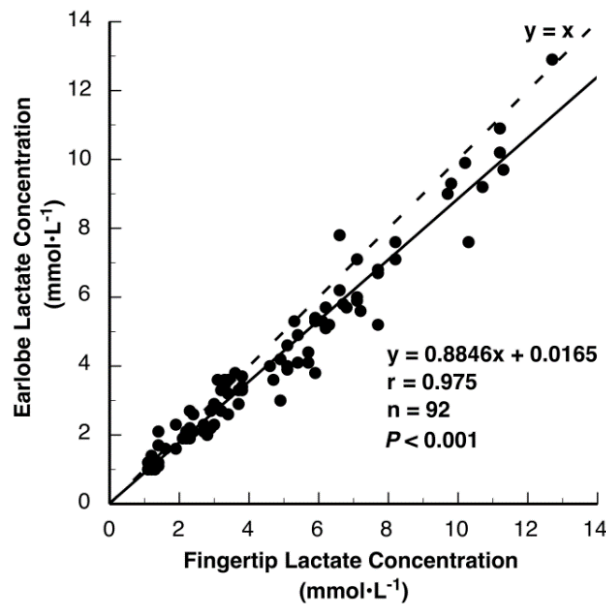


Fig. 1 Relationship of blood lactate concentration between the fingertip and earlobe.

Table 1 Change in blood lactate concentration at the fingertip and earlobe during constant exercise.

		Rest	5 min	10 min	15 min	20 min
45% $\dot{V}O_{2peak}$	Fingertip	0.93 (0.10)	2.10 (0.25)	2.24 (0.28)	2.29 (0.45)	1.95 (0.31)
	Earlobe	1.04 (0.09)	1.86 (0.20)	1.93 (0.25)	1.94 (0.24)	1.88 (0.28)
60% $\dot{V}O_{2peak}$	Fingertip	1.05 (0.05)*	3.99 (0.36)*	4.23 (0.50)	4.01 (0.59)	4.04 (0.57)
	Earlobe	0.83 (0.04)	3.25 (0.28)	3.68 (0.41)	3.84 (0.45)	3.81 (0.45)
75% $\dot{V}O_{2peak}$	Fingertip	0.87 (0.04)	6.80 (0.68)*	8.14 (0.80)*	8.33 (0.87)	8.73 (0.89)
	Earlobe	0.90 (0.04)	5.41 (0.51)	7.06 (0.72)	7.83 (0.86)	7.99 (1.12)

Data are given as the mean (SE). * Significantly higher than the earlobe value ($P < 0.05$).

At 45% $\dot{V}O_{2peak}$, an ANOVA revealed a significant main effect of sampling sites during exercise ($F_{1,7} = 6.777$, $P = 0.035$, $\eta^2 = 0.49$). No significant difference was observed over time ($F_{3,21} = 1.047$, $P = 0.392$, $\eta^2 = 0.13$) nor were significant interactions observed between sampling sites over time ($F_{3,21} = 0.650$, $P = 0.592$, $\eta^2 = 0.08$).

At 60% $\dot{V}O_{2peak}$, an ANOVA revealed a significant main effect of sampling sites ($F_{1,6} = 7.519$, $P = 0.029$, $\eta^2 = 0.52$) and no effect of time ($F_{3,21} = 2.073$, $P = 0.134$, $\eta^2 = 0.23$). A significant interaction between sampling sites over time was observed ($F_{3,21} = 8.364$, $P < 0.001$, $\eta^2 = 0.54$) and a post hoc test indicated that the measurements obtained at the fingertip were significantly higher than those obtained at the earlobe

only at 5 min (MD 0.74 $\text{mmol} \cdot \text{L}^{-1}$, CI 0.43-1.04, $P < 0.05$, $d = 2.01$). There was no significant difference at 10 min (MD 0.54 $\text{mmol} \cdot \text{L}^{-1}$, CI 0.13-0.95, $P > 0.05$, $d = 1.10$), 15 min (MD 0.18 $\text{mmol} \cdot \text{L}^{-1}$, CI -0.35-0.70, $P > 0.05$, $d = 0.28$) or 20 min (MD 0.23 $\text{mmol} \cdot \text{L}^{-1}$, CI -0.13-0.58, $P > 0.05$, $d = 0.53$).

At 75% $\dot{V}O_{2peak}$, an ANOVA revealed a significant main effect of sampling sites ($F_{1,6} = 31.61$, $P = 0.001$, $\eta^2 = 0.84$) and time ($F_{3,18} = 17.89$, $P < 0.001$, $\eta^2 = 0.75$) during exercise. No significant interaction was observed between sampling sites over time ($F_{3,18} = 1.95$, $P = 0.158$, $\eta^2 = 0.25$). A post hoc test indicated that there were significantly higher values obtained in the fingertip than in the earlobe at 5 min (MD 1.39 $\text{mmol} \cdot \text{L}^{-1}$, CI 0.74-2.03, $P < 0.05$, $d = 1.98$) and 10

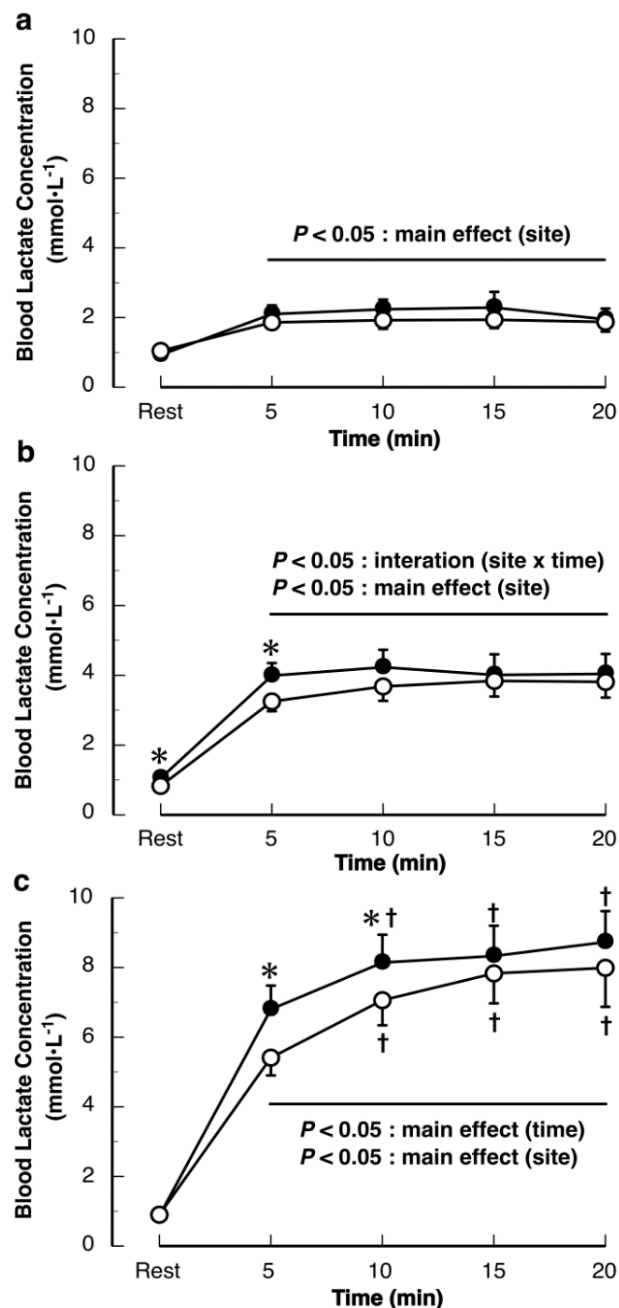


Fig. 2 Change in blood lactate concentration at the fingertip (●) and earlobe (○) during constant exercise at 45% (a), 60% (b), and 75% (c) of peak oxygen uptake.

* significant difference between sampling site ($P < 0.05$). † significant difference after five min at each sampling site ($P < 0.05$).

min (MD 1.09 mmol·L⁻¹, CI 0.31-1.86, $P < 0.05$, $d = 1.30$), but not at 15 min (MD 0.50 mmol·L⁻¹, CI -0.23-1.23, $P > 0.05$, $d = 0.63$) and 20 min (MD 0.74 mmol·L⁻¹, CI 0.06-1.43, $P > 0.05$, $d = 1.00$).

Fig. 3 illustrates the Bland and Altman plot, which is used in a previous study [23], for the 23 paired fingertip and earlobe blood lactate concentrations at

each time. The correlation between mean value of blood lactate concentration and difference were seen at 5 min ($r = 0.846$, CI 0.67-0.93, $P < 0.001$) and 10 min ($r = 0.581$, CI 0.22-0.80, $P = 0.004$), no relationship was observed at 15 min ($r = 0.294$, CI -0.13-0.63, $P = 0.174$) and 20 min ($r = 0.279$, CI -0.15-0.62, $P = 0.197$).

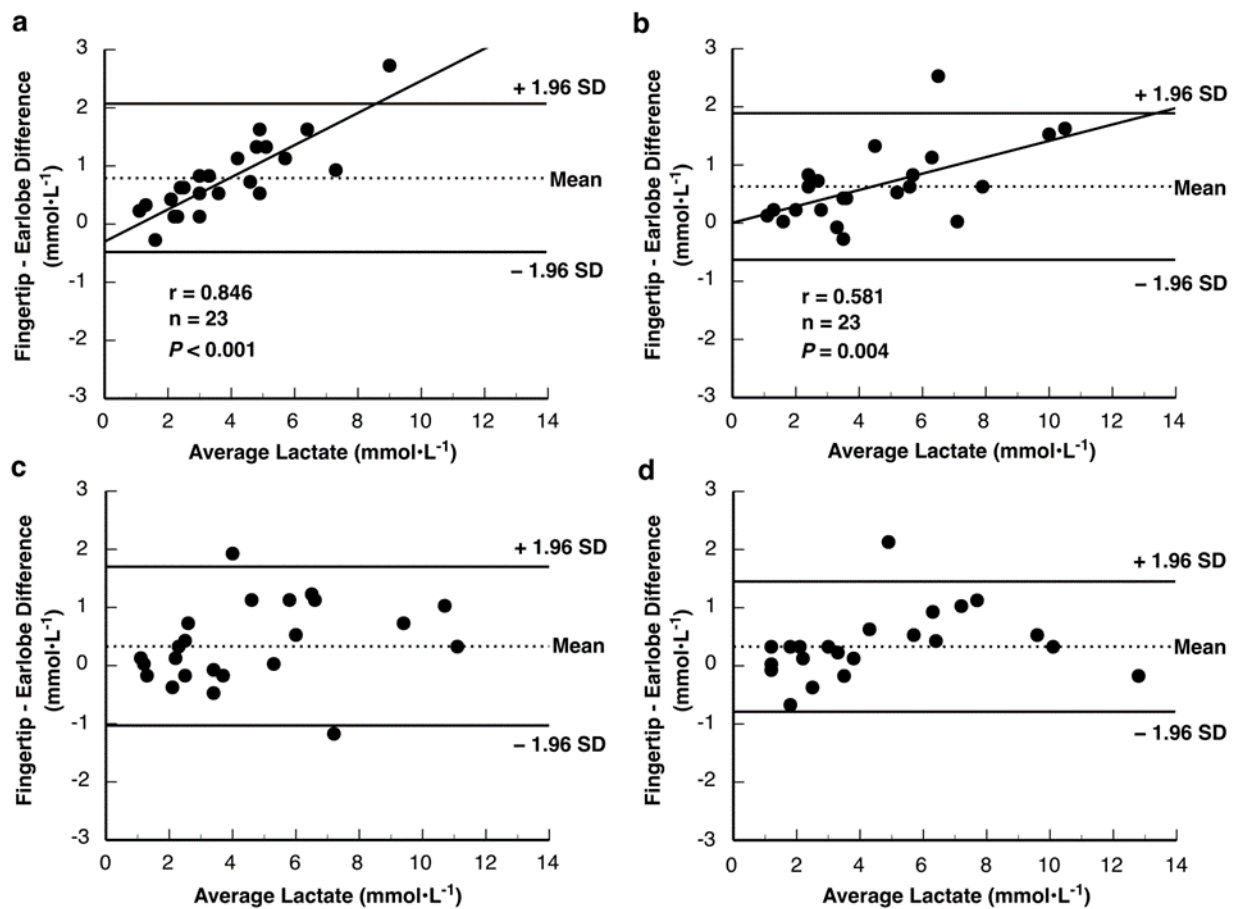


Fig. 3 Limits of agreement plot in blood lactate level between fingertip and earlobe at 5 min (a), 10 min (b), 15min (c), and 20 min (d) during steady state exercise.

4. Discussion

The aim of this study was to determine whether blood sampling sites affect the values of lactate concentrations during steady state cycle exercise. While 92 pairs of blood samples from the fingertip and earlobe indicated a significant correlation, a time course analysis indicated a higher blood lactate level was observed over time in the fingertip than in the earlobe for all three exercise intensities (Fig. 2). Previous studies, which have focused on differences in blood lactate level across sample sites, showed either no significant difference [3, 6, 7] or that the fingertip values tended to be higher than those obtained at the earlobe [4-6]. Our data supports the latter studies. The new finding of the current study was that the difference

in blood lactate level across sampling sites was reduced over time during constant moderate- and high-intensity exercise (Fig. 2). To our knowledge, no studies have examined the effect of time duration on the lactate difference across sampling sites during constant exercise.

It is possible that regional lactate production may affect the change in blood lactate level across sampling sites. Dassonville et al. [5] suggested that gripping the handlebars during cycle exercise can affect the difference in sampling site because of local lactate production. In fact, Wong et al. [20] found that gripping the handlebars produces regional blood lactate. Furthermore, there were less micro circulation effects in blood lactate production. For example, blood flow restriction during exercise elevates the production of

lactate [24]. In moderate bicycle exercise, the finger blood flow decreased until the beginning of about 5 min during a 10-min constant load exercise followed by an increase to resting levels [25]. This data could account for the significant interaction between sampling site and time at moderate-intensity of exercise in this study (Fig. 2b). While blood flow in the finger is reduced at the onset of exercise, it is followed by a low level during heavy exercise [26]. This could be explained by the absence of interaction between sampling sites and time during high-intensity exercise in the present study (Fig. 2c).

Moreover, blood flow reduction could be affected by sympathetic nervous activity, which elicits vasoconstriction. The vessels of peripheral regions that contain fingertip and earlobe are innervated mainly by sympathetic adrenergic vasoconstrictor nerves, not vasodilator nerves [27]. The exercise initiation causes a cutaneous vasoconstriction by noradrenaline secretion from an enhanced sympathetic vasoconstrictor nerve activity [28]. Furthermore, the alterations of norepinephrine level accompanied with blood lactate changes during incremental exercise [29]. Interestingly, the Bland and Altman plot indicated that the sampling site difference in lactate values was increased in a concentration-dependent manner until the first 10 min of exercise (Fig. 3); it reinforces the idea that less micro circulation, following the augment of sympathetic vasoconstriction activity, is associated with the difference across sampling sites in blood lactate at the beginning of exercise. After 15 min of exercise, the rising of internal temperature might evoke the peripheral vasodilation around sampling sites [27], which indicated no such a relationship. We recommend that the earlobe be used for blood lactate evaluation during steady state exercise, because the ear is relatively immune to the vasoconstrictive effects of the sympathetic system compared with the fingertip [12].

A previous study suggested that sweat, which contains a high level of lactate, might affect blood lactate concentration differences across sampling sites

[4]. The hand has a higher local sweat rate [10], which suggests that the fingertip is more impacted by sweating during exercise than is the earlobe. It seems that fingertip levels would remain higher than earlobe levels during exercise, or the difference across sampling site would increase over time. In the present study, the possibility of sweating effects might be low since we obtained contradictory results. However, our experimental environment was controlled for comfort temperature. Consequently, future study is required to determine whether a hot environment would affect to the sampling site difference in blood lactate level.

Blood lactate levels were measured for the evaluation of exercise tolerance in the sports science fields [2]. MLSS has been established as a useful tool for evaluating exercise tolerance [13], which was defined as the highest concentration of blood lactate that can be maintained ($\pm 1.0 \text{ mmol} \cdot \text{L}^{-1}$) during the last 20 min of a 30-min steady state exercise. The results of this study suggest that sampling site differences might affect the value of MLSS. Indeed, if using a verified method, in which the maximal increase of blood lactate concentration is limited to no more than 0.5 mM during the final ten minutes of a 20-min constant load exercise [30], then four participants are within the criteria of MLSS for fingertip evaluation, but not for the earlobe method, during 75% $\dot{V}\text{O}_{2\text{peak}}$ exercise. Further study is needed to determine whether the exercise prescription using MLSS is different across sampling sites.

This study showed that the change in blood lactate concentration during steady state exercise is different between sampling sites. However, there are some limitations to this study. First, we cannot suggest which sampling site has a precise value of lactate. Further study is needed to show the accurate sampling site in comparing with evaluation of arterial blood sampling. Second, our study examined only young subjects. MLSS has not been well investigated during the aging process [31]; thus, this needs to be examined in future studies. Considering that older adults exhibit more extensive vasoconstriction in the non-active limb than

young subjects during exercise [32], the lactate difference across sampling sites should be investigated in older subjects. Third, the sample size was smaller than in previous studies that assessed sampling site difference, $n = 312$ [5], $n = 45$ [6], and $n = 26$ [4, 14]. However, other some studies have made use of a small study population: $n = 7$ [33], $n = 8$ [34], and $n = 9$ [3]. Therefore, it is likely that size does not affect the main results of this study.

5. Conclusions

This study demonstrated that blood lactate concentration indicated different changes according to sampling site during steady-state cycling exercise. The measurements obtained in the fingertip tended to be higher than those obtained in the earlobe; however, the differences became smaller over time, particularly during moderate- and high-intensity constant cycle ergometry. These results could indicate that blood lactate evaluation of constant exercise capacity (i.e., MLSS) is affected by the lactate sampling site. In future MLSS studies, sampling sites should be considered. This study shows that using earlobe sampling could be better for measurement of blood lactate concentration during constant exercise.

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