Lactate Dynamics in Skin Interstitial Fluid Predicts Lactate Threshold in Exercising Rats

Shohei Dobashi[1,2,5], Daisuke Funabashi[1,3,5], Kazuki Sameshima[1], Noriko Tsuruoka[4], Takashi Matsui[1,2,3]*

[1] Exercise Biochemistry & Sport Neurobiology Division, Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan

[2] R&D Center for Lifestyle Innovation, University of Tsukuba, Tsukuba, Japan

[3] Advanced Research Initiative for Human High Performance, University of Tsukuba, Tsukuba, Japan

[4] Graduate School of Biomedical Engineering, Tohoku University, Sendai, Japan.

[5]These authors contributed equally to this work.

Correspondence:
Takashi Matsui Ph.D.
Assistant Professor
Exercise Biochemistry & Sport Neurobiology Division,
Institute of Health and Sport Sciences, University of Tsukuba
E-mail: matsui.takashi.ga@u.tsukuba.ac.jp Tel: +81-29-853-2750

Abstract

The blood lactate threshold (LT) is a crucial marker of metabolic shifts and stress transitions during exercise, influencing optimal intensity determination and chronic exercise impact evaluation across diverse individuals. Although recent studies have explored methods for estimating blood lactate levels without blood sampling, accurate solutions for LT estimation remain undeveloped. Here, we tested the analytical capability of an ultra-minimally invasive technique for LT measurement that harnesses skin interstitial lactate dynamics using a single microneedle perfusion system. The rats were acclimated to exercise and underwent external jugular vein cannulation. Following comprehensive recovery, a 1 mm single microneedle was implanted into the back skin. This allowed for the simultaneous measurement of lactate concentrations in the blood and skin interstitial fluid during incremental treadmill exercise. Skin lactate progressively increased in tandem with blood lactate during incremental exercise, albeit with lingering post-exercise clearance that did not correlate with blood lactate. Remarkably, LT extrapolated from skin lactate showed significant alignment with blood LT. Our findings reveal the potential of skin interstitial dynamics to accurately assess LT during exercise using a single microneedle, integrating the discoveries of exercise physiology into real-world athletic and/or healthy practices.

Keywords

Exercise, Lactate, Blood, Skin interstitial fluid, Single microneedle, Rat

Introduction

In the field of sports science, where the enhancement of athletic performance and promotion of physical well-being are paramount objectives, the evaluation of exercise intensity and assessment of chronic exercise impact assumes a pivotal role. Traditionally, blood lactate concentration has been used as a fundamental physiological marker, with particular emphasis on its dynamic behavior during periods of rest, exercise, and recovery. In this context, universal indices that denote aerobic and anaerobic work capacities encompassing the lactate threshold (LT), peak lactate concentration, and maximal lactate steady state (MLSS) during constant and incremental load exercises have been developed and used in a diverse population of athletes and exercise enthusiasts[1,2]. Hence, continuous, real-time assessment of blood lactate concentration during exercise is important for the development of sports medicine and science. However, continuous blood sampling from the insertion of indwelling needles [3,4] or the ear lobe and the fingertip are required to detect LT during exercise[5]. Therefore, despite the acknowledged significance of continuous blood lactate monitoring during exercise, there is a considerable gap between theoretical recognition and practical applications.

In recent sports science, there has been a noteworthy surge in the rapid exploration of alternative biomarkers as replacements for blood lactate concentration, along with the development of robust quantitative assessment methodologies. Devices such as mouthpieces, eyewear, and tattoo-seal-types have emerged as non-invasive platforms for the collection of saliva and sweat lactate[6-8]. Seki et al. confirmed that LT calculated from sweat lactate levels during exercise was significantly associated with LT derived from blood lactate concentrations[9]. However, the same research group also reported that there were some cases in which sweat LT failed to align with blood LT, attributable to individual variations in sweat patterns[10]. Moreover, the evaluation of sweat lactate cannot be performed in scenarios where exercise occurs in cold environments, during swimming activities, or in low-intensity workouts, where sweat production is minimal or infrequent.

We then focused our attention on the skin interstitial fluid (ISF). The primary constituents of ISF are plasma, and these interact at the capillaries, which are permeable and allow water and small polar solutes to interchange freely between the two fluids by diffusion[11]. Moreover, as the blood capillary hydrostatic pressure is higher than that of the ISF, advective flow of solution-contained water from plasma are encouraged to ISF[11]. Thus, especially, low-molecular-weight substances such as blood lactate and blood glucose are believed to exhibit profiles in skin tissue fluid similar to their blood concentrations, as they can diffuse across vessel walls[12,13]. Indeed, previous studies have reported correlations between the dynamics of glucose and lactate concentrations in human skin ISF and the blood[14,15]. More recently, systems have been developed

that allow rapid and continuous monitoring of skin ISF lactate as an electrical current using enzyme electrodes within wearable sensors[16,17]. Furthermore, a recent investigation has begun to investigate the dynamics in skin ISF lactate during exercise and has shown that the dynamics of that are similar to those of the blood lactate concentrations[18]. However, many previous systems collecting skin ISF had to take much time to collect sufficient volume of that, because the high resistivity of the dermis to fluid flow limits both sampling rate and volume of ISF[19]. This limitation hinders the evaluation of the dynamics of the components in ISF. Moreover, it has been reported that there is a time lag between the variations in lactate in the skin interstitial fluid during exercise and those in the blood lactate concentration[20,21]. It has been mentioned that this time lag may be due to a delay in the collection of interstitial fluid because of the spread of the wound around the indwelling needle during exercise and the time it takes for the interstitial fluid to reach the needle.[20,21]. Recent wearable devices have microneedles which length is about 5-13 mm,[22] and/or many of them have been designed with multiple projections[11]. This can potentially increase user discomfort during prolonged physical activity and may lead to the expansion of sensor insertion site wounds, thereby introducing time delays in the immediate monitoring of lactate levels in the skin interstitial fluid.

In contrast, Tsuruoka et al. designed a single ultra-minimally invasive micro needle with an outer diameter of approximately 0.2 mm and a length of 1-2 mm[23], which seems to be less invasive than conventional microneedles. Moreover, Tsuruoka's system collects ISF by perfusing saline solution, which dilutes the concentration of components, but allows continuous collection of the volume of solution needed to measure the contents. Indeed, the same authors have investigated the effectiveness of the microneedle perfusion systems by administering a lactate solution to mice with the microneedle inserted, and lactate concentrations were quantified directly outside the body using a lactate analysis device. The results indicated a close temporal alignment between post-lactate administration blood lactate concentrations and skin fluid lactate concentrations with a significant positive correlation. Additionally, the range of blood lactate concentrations that exhibited a significant correlation with skin fluid lactate concentrations at this time exceeded the onset of blood lactate accumulation (OBLA) (4 mM), suggesting that the microneedle perfusion system of Tsuruoka et al. may offer the potential to predict LT from skin ISF lactate levels during incremental load exercises[23]. Such a system appears to minimize the impact of enzyme electrode drawbacks, which are susceptible to interference due to prolonged wear[24], while significantly reducing the user's burden during LT evaluation. However, it remains uncertain whether lactate collected in the skin ISF through the microneedle perfusion system accurately reflects the LT during incremental load exercises.

Here, we provide the first scientific evidence of the analytical capability of an ultra-minimally invasive technique for LT measurement that harnesses skin interstitial lactate dynamics using a

single microneedle perfusion system. Previous studies have investigated the possibility of sweat contamination in ISF samples collected from the epidermis[25] as well as the potential negative effects on exercise performance due to repeated blood sampling during traditional incremental exercise, leading to increased pain and stress[26]. To address these concerns, we conducted incremental exercise in rats without sweat production in the epidermis. The results of this study suggest the potential use of skin ISF lactate as an effective alternative to current methods for measuring blood lactate levels during exercise, offering groundbreaking, world-first evidence with the potential to significantly contribute to the advancement of sports medicine and exercise science research.

Results

Lactate concentration increases in the skin fluid and blood during incremental treadmill running.

Following running habituation, the rats with inserted jugular catheters and single microneedles were subjected to incremental running on the treadmill (Fig. 1*A*-*C*). The treadmill running speed was gradually increased every 3 min until exhaustion (running time:34.4 ± 1.9 min; peak speed:40.3 ± 1.6 m/min). Blood lactate concentrations significantly increased as running speed increased, reaching 8.2 ± 0.8 mM at exhaustion (Fig. 2*A*). Following the exercise test, the blood lactate concentrations rapidly decreased to baseline levels. Skin fluid lactate concentrations also gradually increased during incremental treadmill running but did not decrease to baseline levels even 30 min after the exercise test (Fig. 2*B*). We confirmed that any fluid leaked subcutaneously or into the skin after removing the microneedle (Fig. 1*D*), and the amount of skin fluid collected did not change among the time points (Fig. 2*C*).

Skin fluid lactate dynamics were significantly associated with blood lactate dynamics during the exercise test but not afterward.

We then examined the relationship between lactate dynamics in blood and skin fluid (Fig. 3). A very high correlation was observed between them in individual rats (Fig. 3*A*-*B*). The pooled analysis also revealed a moderately significant correlation coefficient (Fig. 3*C*). However, these significant correlations disappeared in the recovery phase following the exercise test in both individual and pooled analyses (Fig. 3*D*-*F*).

Lactate threshold calculated from skin fluid lactate are closely associated with those calculated from blood.

We attempted to calculate LT derived from skin fluid and examined its validity by performing a

correlation analysis with LT calculated from blood lactate dynamics. First, we performed LT determination using the *V-slope* method[27], calculating the running speed and lactate levels in the blood and skin fluid separatory (Fig. 4). The absolute and relative (% peak) running speed and lactate levels corresponding to each LT calculated from blood and skin fluid were similar in typical participants (Fig.4*A-D*). Moreover, no significant differences were observed in the absolute and relative running speeds corresponding to the LT determined from the blood and skin fluids (Fig. 5*A, C*). Furthermore, we confirmed that skin LT was significantly associated with blood LT levels (Fig. 5*B, D*).

Discussion

This study tested the analytical capability of an ultra-minimally invasive technique for LT measurement that harnesses skin interstitial lactate dynamics using a single microneedle perfusion system (Fig. 1). Our results show that lactate levels in rat skin fluid collected using a single microneedle perfusion system increased during incremental treadmill running, which was significantly associated with an increase in blood lactate levels (Figs. 2-3). We also confirmed that the LT determined from the blood and skin fluids were comparable and closely related (Figs. 4-5). As we discovered these findings in rats without sweat production in the epidermis, our results strictly proved that skin ISF can be applied for LT prediction during exercise (Supplemental fig.1).

Validity of the rat model for incremental exercise to determine lactate threshold.

The incremental exercise gradually increased blood lactate concentration in rats, and the LT calculated by *V-slope* method[27] revealed 22.8 ± 0.8 m/min (absolute running speed) and 56.4 ± 2.2% (relative to the running speed at exhaustion) (Fig. 5*A, C*). We conducted an exercise test using an experimental protocol modified from a previous study, and both the absolute and relative LT were higher than those in the study[28]. Although our study used rats that conducted only running habituation without any specific training, the relative LT was similar to that of untrained humans (approx. 50-60% peak speed)[29]. Hence, the incremental exercise model in the present study seems to be a reasonable experimental model for simulating LT determination using the human incremental exercise test.

Lactate dynamics in the perfusate from the epidermal tissue are correlated with blood during exercise.

Notably, skin fluid lactate concentration increases as well as blood lactate during the exercise test (Fig. 2*A-B*). Moreover, lactate dynamics in skin fluid were significantly associated with those in blood (Fig. 3*A-C*). These results are in line with those of a previous study that investigated the

relationship between blood and skin fluid lactate in resting mice after intraperitoneal injection of lactate solution[23]. Since the components (at least the concentrations of relatively smaller molecules such as glucose and lactate) of ISF are comparable to the plasma[11,12], our results indicate that the lactate levels in perfusate reflect the concentration in skin ISF, and the microneedle perfusion system successfully collected skin ISF even during exercise. Thus, the microneedle perfusion system might be effective in assessing lactate dynamics even during exercise. Previous studies have demonstrated that lactate dynamics in the skin ISF during and after exercise is delayed compared to that in the blood[20,21]. However, the lactate concentrations in skin ISF in this study were related to those in blood at time points that were assumed to correspond theoretically, suggesting that the measurements were accomplished without a time lag. Previous studies have suggested that wound widening due to continuous invasive needle placement can cause a time delay in lactate monitoring by preventing the collection of ISF [20,21]. In this context, extremely low invasiveness of the microperfusion systems that consist of only a single microneedle might make continuous lactate motoring without a time lag (Fig. 1*B-D*). In addition, skin blood flow increases during exercise[30], which might also enable lactate measurement without delay possibly via facilitating the diffusion of water-soluble constituents from the blood to the skin. From these findings, we speculate that the ultra-minimally invasive systems and the increase in skin blood flow realized the lactate monitoring during exercise without time delay in this study. Furthermore, the lactate concentrations in perfusates collected in vitro were directly quantified by the fluorescent staining method[23]. Collectively, we believe that the present study strictly proved the relationship between blood and skin fluid lactate during exercise.

While a high positive correlation of lactate dynamics during exercise was observed between blood and skin fluid within each individual rat, the correlation coefficient of the pooled analysis decreased to moderate levels (but it was still statistically significant). The reason for this is assumed to be that the slope of the regression line between blood and skin fluid lactate levels was different for each rat. Although it may be possible to estimate the responsiveness of blood lactate dynamics from skin fluid dynamics, absolute quantification of blood lactate concentration might be currently difficult based on skin fluid lactate dynamics.

The lactate thresholds calculated from the blood and skin fluid are closely correlated.

Both the absolute values of LT calculated from the blood and skin perfusate and their relative values to peak speed were not significantly different (Fig. 5*A,C*). Furthermore, there was an extremely strong positive correlation between the two (Fig. 5*B, D*). Accordingly, LT can be estimated from the dynamics of skin ISF during exercise. A previous study pointed out that ISF collected from the epidermal tissue may be contaminated by sweat[22]; therefore, we used rats that did not produce sweat from the epidermis as subjects in this study. From these observations, this

study may be the first to directly prove the effectiveness of skin fluid LT as an alternative marker of blood LT.

Furthermore, the skin fluid LT corresponded to 50-65 % of peak speed as well as blood LT, and the results of the correlation analysis suggested that the individual differences were clearly captured in that range (Fig. 5*D*). Exercise at moderate intensity including 50-65% of maximal intensity, is widely used in training programs for amateur-level athletes and health-enhancing exercisers[27], and it has been suggested that LT assessed by skin fluid may be applicable in many sports settings. Future studies should examine the validity of the system used in this study for highly athletic individuals with LT corresponding to 70-90% of maximal effort[31].

The lactate levels in the skin fluid did not decrease to baseline levels after the exercise test. Interestingly, the increase in blood lactate concentration rapidly decreased to baseline levels (Fig. 2*A*), but those in the skin fluid decreased slowly and remained significantly higher 30 min after the completion of the exercise test (Fig. 2*B*). Additionally, the significant relationship of the lactate dynamics between the blood and skin fluid observed during exercise disappeared during the recovery phase after exercise (Fig. 3*D-F*). Since Ming et al. confirmed a slow reduction in skin ISF lactate concentrations after acute exercise in humans, our results support this response[17]. The authors speculated that the reason for this is that changes in components in the ISF accompanied by a relative reduction in the perfusion rate of ISF to the skin after exercise might be involved[17]. However, if the flow rate had been decreased, the skin fluid lactate concentrations would have been diluted in saline circulating in the microneedle perfusion system. Thus, the relatively high concentrations of skin fluid lactate after the exercise test; nevertheless, almost the same amount of skin perfusate was collected, suggesting that clearance of lactate in the skin tissue may have been slower than that in skeletal muscles and the blood rather than the reduction in perfusion rate of ISF. This explanation should be supported by a rapid post-exercise decrease in skin blood flow[30], which affects the clearance of water-soluble substances from the skin into the blood[32].

Growing evidence suggests that lactate induces several physiological adaptations via its receptor (i.e., G-protein coupled receptor 81) in various tissues[33,34]. In addition, a recent study suggested that regular exercise rejuvenated aging skin conditions[35]. A recent review highlighted that lactate acts as a signaling molecule that interacts with mitochondrial biogenesis and turnover and tunes the ability of cells to respond to stressors[36]. Therefore, these physiological adaptations in skin tissue might occur due to lactate accumulation after exercise. While further investigations should be required for testing the hypothesis, the microneedle perfusion system used in this study might be a great tool for addressing it.

Perspectives

Collectively, our findings indicate that skin fluid lactate reflects the dynamics of blood lactate during exercise and may be a novel valuable biomarker to estimate LT with high accuracy. This suggests that LT could be assessed without the need for blood sampling by the single microneedle perfusion system. However, since the dynamics following the exercise tests were different between the blood and skin fluid, it seems difficult to assess the lactate clearance ability after exercise and lactate clamp[37]. Moreover, we had measured lactate concentrations in perfusate, collected in vitro, after the completion of exercise test. Thus, the current microneedle perfusion system could not provide immediately and continuous feedback. In the future, further miniaturization and development of sensors that enable immediate evaluation are needed for human applications.

Nonetheless, it is noteworthy that the micro perfusion system is theoretically capable of evaluating the dynamics of other metabolites and endocrine products (e.g., ketones, testosterone, corticosterone, growth hormone, and so on) as well as lactate. Hence, the experimental systems may be able to observe the dynamics of secretion and/or metabolites during exercise and daily living including sleep, which may shed light on the development of mechanistic investigations in the field of exercise physiology and biochemistry. Moreover, as lactate levels are related to the criteria for sepsis[38]; therefore, the current microneedle perfusion system may also contribute the medical field such as early detection for sepsis and other diseases.

Limitations

This study has some limitations. First, the time resolution of the lactate assessment in skin perfusate was low. The microneedle perfusion system used in this study required a flow rate of 5.0 μl/min in the tubes to ensure a stable ISF amount[20]. Moreover, a minimum of 20 μl of perfusate was required for the quantification of lactate concentrations, so the collection frequency was less than that of blood. Thus, insufficient skin perfusate collection, especially at the running speed close to all-out, to collect enough amount to calculate LT2, the point at acceleration of lactate accumulation[1,2]. LT2 is an index suggesting that the exercise intensity is physiologically high and is particularly important in training elite athletes[1,2]. However, we have observed some cases in where the rat could keep running even high-intensity were able to collect sufficient points of skin perfusate to calculate LT2 (Supplemental Fig. 2). Accordingly, the time resolution should be improved to enhance accurate measurements of skin fluid lactate concentrations at various exercise intensities. Second, this study did not perform the analysis of ventilatory responses. Ventilatory threshold (VT) is also used to assess the physiological exercise intensity in the training field, and a previous study in where the LT calculated using sweat lactate was significantly associated with the VT in human subjects[9]. While it is classically known that LT and VT

approximate each other[1,2], inclusion of ventilatory gas analysis would further enhance the physiological validity of LT determination using the skin fluid. At last, we discussed the possibility that the changes in skin blood flow might relate to the skin ISF lactate dynamics, nevertheless we did not measure it in this study. Future studies should measure skin blood flow during exercise to address this hypothesis.

Conclusion

In this study, we tested the analytical capability of an ultra-minimally invasive ISF collecting technique using a single microneedle perfusion system on LT measurements in exercising rats. Our findings reveal the potential of skin interstitial dynamics to accurately assess LT during exercise using a single microneedle. Currently, our group is challenging to develop a wearable sensor capable of real-time measurement with the aim of applying this system to humans. The implementation of this system may lead to the construction of various training programs and evaluation of training effects through the evaluation of not only LT but also MLSS. We believe that our findings will provide valuable insights for integrating the discoveries of exercise physiology into real-world athletic and/or healthy practices.

Methods

Animals and running habituation.

The study was performed with the approval of the University of Tsukuba Animal Experiment Committee in accordance with the institutional guidelines of the University of Tsukuba and in compliance with the ARRIVE guidelines. Adult male Wistar rats (250–300 g) (SLC), housed and cared for in an animal facility, were fed a standard pellet diet (MF; Oriental Yeast) and provided water ad libitum. Room temperature was maintained between 22 and 24 °C under a 12-h light–12-h dark cycle (lights on, 0700 to 1900 h). The rats were habituated to running on a treadmill (Natsume, Tokyo, Japan) for five sessions over 6 days for 30 min/day. The running speed was gradually increased from 5 to 25 m/min[39-41].

Surgery to insert jugular catheter.

After habituation to treadmill running, the rats were anesthetized with isoflurane and a silicone catheter was inserted into the jugular vein and fixed with a silk thread suture (32 mm). The external distal end of the catheter was fixed at the nape of the neck. Subsequent experiments and analyses were carried out 2 d after surgery[39-41].

Inserting the ultra-minimally invasive microneedle and incremental treadmill running test. Two days after the surgery to insert catheter, the ultra-minimally invasive microneedle was inserted into the back skin of the rats under anesthetization 30 min before start of the exercise test. The specification of the microneedle was previously described[20]. To place the needle in the subepidermal tissue, the needle was inserted along the skin surface (Fig. 1B). The inlet syringe was filled with saline as the perfusate. Perfusion at a flow rate of 5 μl/min was started immediately after the needle was secured to the rat's back. This flow rate was determined considering the efficient collecting perfusate (skin ISF mixture saline)[20]. After the completion of inserting microneedle into the back skin in the rats under anesthesia, the rats were moved on the treadmill and ends of the tubes were connected to syringes attached to push (ESP-36, Eicom, Kyoto, Japan) and pull (Nexus 3000, Chemyx Inc., USA) syringe pumps. 25-30 min after waking up, the animals were further kept rest for 18 min before the running test (Fig. 1A). The initial treadmill velocity was 5 m/min and was increased 5 m/min every 3 min until the speed at 20 m/min. Thereafter, the treadmill speed was increased 2.5 m/min every 3 min until the point of exhaustion. Exhaustion was considered to have occurred when the rat was unable to keep pace with the treadmill and stayed on the grid positioned at the back of the treadmill for a period of 30 s despite being gently pushed with sticks or breathed on. This protocol is modified from an incremental treadmill exercise protocol to determine blood LT for rats[25]. Following the reached exhaustion, the rats were kept rest for 35 min on the treadmill.

Blood and skin fluid sampling.

Blood samples were collected from the catheter every 3 min; however, it was collected 30 seconds before each speed increase to determine blood LT during the exercise test. Due to the specification of the microneedle perfusion system in this study, perfusing at 5 μl/min would result in a time delay of 5 min compared to a real concentration change of skin ISF. Moreover, as the assay kit for detecting the lactate concentration needed at least 20 μl of perfusate per sample, we collected perfusate every 6 min, the collection was conducted 5 min after blood sampling from the catheter (Fig. 1*C*). Theoretically, the components in the collected perfusate reflects the average values of 3 points of blood samples (5, 8, and 11 min before the skin fluid sampling). Therefore, the collected perfusate at 35 min after the exhaustion reflects until 30 min after the exercise test. In a previous study, it is suggested that the flow rate of perfusate could be changed during the recovery phase after acute exercise[17]. Thus, we measured collected amount of the skin fluid in this study. We also confirmed that any fluid leaked subcutaneously or into the skin after removing the microneedle (Fig. 1D).

Lactate measurement and determination of lactate threshold.

Blood lactate levels were measured using an automated lactate analyzer (YSI 2500, YSI Inc., Yellow Springs, USA). Skin fluid lactate levels were determined using a commercial fluorescent stain kit (L-Lactate Assay Kit, Cayman Chemical Company, USA), according to the manufacturer's instructions. Fluorometric determination was performed using a fluorescence microplate reader (Varioskan LUX multimode microplate reader, Thermo Fisher Scientific, USA).

LT was determined using the V-slope method[24], calculating the running speed corresponding to the intersection of two linear regression lines derived separately from the data points of the running speed and the lactate level.

Statistical analysis.

One-way analysis of variance (ANOVA) was performed for the time course changes in blood and skin fluid lactate concentrations, and skin fluid sample amount. Pearson's correlation analysis between the lactate concentrations in blood and skin samples was conducted separately during and following the exercise test. As mentioned above, the collected skin fluid might be reflected the 3 points of blood samples due to the methodological specification; therefore, we conducted the correlation analysis using the average concentration of 3 blood lactate per 1 skin fluid lactate. We also compared the absolute and relative (% peak) treadmill speed corresponding to the LT calculated from the blood and skin by paired t-test and examined their relationships between the LT calculated from blood and skin fluid by Pearson's test. All statistical analysis was performed using the GraphPad Prism ver. 10.0.3 (MDF, Japan).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

This research was supported in part by the Leading-edge Research Project for Sports Medicine and Science (LRP) by Japan Sports Agency to T.M., Grant-in-Aid for Scientific Research (B) by JSPS KAKENHI (19H03994 and 22H03478) to T.M., and Fusion Oriented Research for disruptive Science and Technology (FOREST) by Japan Science and Technology Agency (JST) to T.M. (JPMJFR205M) and N.T.(JPMJFR215).

Author contributions

T.M. and N.T. conceived and designed the study. N.T. prepared a microneedle for the collection of subepidermal interstitial fluid. D.F., S.D., K.S., and T.M. collected data. D.F. and S.D.

determined the blood and skin lactate concentrations. K.S. measured skin fluid amount. D.F. and T.M. conducted data analysis and interpreted the data. S.D., T.M., and D.F. drafted the manuscript. All authors edited and revised the manuscript, and read and approved the final version of this manuscript.

Competing interests

The authors declare no competing interests.

References

1. Poole, D., Rossiter, H.B., Brooks, G.A., and Gladden, L.B. (2021). The anaerobic threshold: 50+ years of controversy. J. Physiol. *599*, 737-767. https://doi.org/10.1113/JP280980.

2. Jaminick, N.A., Pettitt, R.W., Granata, C., Pyne, D.B., and Bishop, D.J. (2020). An examination and critique of current methods to determine exercise intensity. Sports Med. 50, 1729-1756. https://doi.org/10.1007/s40279-020-01322-8.

3. Smith, E.W., Skelton, M.S., Kremer, D.E., Pascoe, D.D., and Gladden, L.B. (1997). Lactate distribution in the blood during progressive exercise. Med. Sci. Sport. Exerc. 29, 654-660. https://doi: 10.1097/00005768-199705000-00011.

4. Goodwin, M.L., Harris, J.E., Hernández, A., and Gladden, L.B. (2007). Blood lactate measurements and analysis during exercise: a guide for clinicians. J. Diabetes Sci. Technol. 1, 558-569. https://doi.org/10.1177/193229680700100414.

5. Robergs, R.A., Chwalbinska-Moneta, J., Mitchell, J.B., Pascoe, D.D., Houmard, J., and Costill, D.L. (1990). Blood lactate threshold differences between arterialized and venous blood. Int. J. Sports Med. 11, 446-451. https://doi.org/10.1055/s-2007-1024835

6. Kim, J., Valdés-Ramírez, G., Bandodkar, A.J., Jia, W., Martinez, A.G., Ramírez, J., Mercier, P., and Wang, J. (2014). Non-invasive mouthguard biosensor for continuous salivary monitoring of metabolites. Analyst 139, 1632-1636. https://doi.org/10.1039/C3AN02359A.

7. Jia, W., Bandodkar, A.J., Valdés-Ramírez, G., Windmiller, J.R., Yang, Z., Ramírez, J., Chan, G., and Wang, J. (2013). Electrochemical tattoo biosensors for real-time noninvasive lactate monitoring in human perspiration. Anal. Chem. 85, 6553-6560. https://doi.org/10.1021/ac401573r.

8. He,W., Wang, C., Wang, H., Jian, M., Lu, W., Liang, X., Zhang, X., Yang, F., and Zhang, Y. (2019). Integrated textile sensor patch for real-time and multiplex sweat analysis. Sci. Adv. 5, eaax0649. https://doi.org/ 10.1126/sciadv.aax0649.

9. Seki, Y., Nakashima, D., Shiraishi, Y., Ryuzaki, T., Ikura, H., Miura, K., Suzuki, M., Watanabe, T., Nagura, T., Matsumoto, M., et al. (2021). A novel device for detecting anaerobic threshold using sweat lactate during exercise. Sci. Rep. 11, 4929. https://doi.org/10.1038/s41598-021-

84381-9.

10. Maeda, Y., Okawara, H., Sawada, T., Nakashima, D., Nagahata, J., Fujitsuka, H., Ikeda, K., Hoshino, S., Kobari, Y., Katsumata, Y., et al. (2023). Implications of the onset of sweating on the sweat lactate threshold. Sensors 23, 3378. https://doi.org/ 10.3390/s23073378.

11. Heikenfeld, J., Jajack, A., Feldman, B., Granger, S.W., Gaitonde, S., Begtrup, G., and Katchman, B.A. (2019). Assessing analytes in biofluids for peripheral biochemical monitoring. Nat. Biotechonol. 37, 407-419. https://doi.org/ 10.1038/s41587-019-0040-3.

12. Scallan, J., Huxley, V.H., and Korthuis, R.J. (2010). Capillary fluid exchange: regulation, functions, and pathology. Chaptor 2 The Interstitium. In Integrated Systems Physiology: from Molecule to Function to Disease (Ganger DN and Granger JP, eds), Morgan & Vlaypool Life Sciences. https://doi.org/10.4199/C00006ED1V01Y201002ISP003

13. Miller, P.R., Taylor, R.M., Tran, B.Q., Boyd, G., Glaros, T., Chavez, V.H., Krishnakumar, R., Sinha, A., Poorey, K., Williams, K.P., et al. (2018). Extraction and biomolecular analysis of dermal interstitial fluid collected with hollow microneedles. Commun. Biol. 1, 173. https://doi.org/10.1038/s42003-018-0170-z.

14. Petersen, L.J. (1999). Interstitial lactate levels in human skin at rest and during an oral glucose load: a microdialysis study. Clin. Physiol. 19, 246-250. https://doi.org/10.1046/j.1365-2281.1999.00174.x.

15. Enderle, B., Moser, I., Kannan, C., Schwab, K.O., and Urban, G. (2018). Interstitial glucose and lactate levels are inversely correlated with the body mass index: need for in vivo calibration of glucose sensor results with blood values in obese patients. J. Diabetes Sci. Technol. 12, 341-348. https://doi.org/10.1177/1932296817730377.

16. Wang, Y., Aursi, I.R., Wang, Z., Derry, C., and Tang, X.S. (2020). Towards a transdermal membrane biosensor for the detection of lactate in body fluids. Sens. Actuators B Chem. 308, 127645. https://doi.org/10.1016/j.snb.2019.127645.

17. Chien, M.N., Fan, S.H., Huang, C.H., Wu, C.C., and Huang, J.T. (2022). Continuous lactate monitoring system based on percutaneous microneedle array. Sensors 22, 1468. https://doi.org/10.3390/s22041468.

18. Ming, D.K., Jangam, S., Gowers, S.A.N., Wilson, R., Freeman, D.M.E., Boutelle, M.G., Cass, A.E.G., O'Hare, D., and Holmes, A.H. (2022). Real-time continuous measurement of lactate through a minimally invasive microneedle patch: a phase I clinical study. BMJ Innov. 8, 87-94. http://dx.doi.org/10.1136/bmjinnov-2021-000864.

19. Friedel, M., Thompson, I.A.P., Kasting, G., Polsky, R., Cunningham, D., Soh, H.T., and Heikenfeld, J. (2023). Opportunities and challenges in the diagnostic utility of dermal interstitial fluid. Nat. Biomed. Eng. https://doi.org/10.1038/s41551-022-00998-9. Online published Jan 19, 2023.

20. de Boer, J., Plijter-Groendijk, H., Visser, K.R., Mook, G.A., and Korf, J. (1994). Continuous monitoring of lactate during exercise in humans using subcutaneous and transcutaneous microdialysis. Eur. J. Appl. Physiol. Occup. Physiol. 69, 281-286. https://doi.org/10.1007/BF00392031.

21. Ellmerer, M., Schaupp, L., Trajanoski, Z., Jobst, G., Moser, I., Urban, G., Skrabal, F., and Wach, P. (1998). Continuous measurement of subcutaneous lactate concentration during exercise by combining open-flow microperfusion and thin-film lactate sensors. Biosens. Bioelectron. 13, 1007-1013. https://doi.org/10.1016/s0956-5663(98)00002-5.

22. Chien, M.N., Chen, Y.J., Bai, C.H., and Huang, J.T. (2022). Continuous glucose monitoring system based on percutaneous microneedle array. Micromachines.13, 478. https://doi.org/10.3390/mi13030478.

23. Tsuruoka, N., Ishii, K., Matsunaga, T., Nagatomi, R., and Haga, Y. (2016). Lactate and glucose measurement in subepidermal tissue using minimally invasive microperfusion needle. Biomed. Microdevices 18, 19. https://doi.org/10.1007/s10544-016-0049-z.

24. Rathee, K., Dhull, V., Dhull, R., and Singh, S. (2015). Biosensors based on electrochemical lactate detection: A comprehensive review. Biochem. Biophys. Rep. 5, 35-34. https://doi.org/10.1016/j.bbrep.2015.11.010.

25. Kim, J., Sempionatto, J.R., Imani, S., Hartel, M.C., Barfdokht, A., Tang, G., Campbell, A.S., Mercier, P.P., and Wang, J. (2018). Simultaneous monitoring of sweat and interstitial fluid using a single wearable biosensor platform. Adv. Sci. 5, 1800880.

https://doi.org/10.1002/advs.201800880.

26. Xuan, X., Chen, C., Molinero-Fernandez, A., Ekelund, E., Cardinale, D., Swarén, M., Wedholm, L., Cuartero, M., and Crespo, G.A. (2023). Fully integrated wearable device for continuous sweat lactate monitoring in sports. ACS Sens. 8, 2401-2409. https://doi.org/10.1021/acssensors.3c00708.

27. Beaver, W.L., Wasserman, K., and Whipp, B.J. (1986). A new method for detecting anaerobic threshold by gas exchange. J. Appl. Physiol. 60, 2020-2027. https://doi.org/10.1152/jappl.1986.60.6.2020.

28. Soya, H., Mukai, A., Deocaris, C.C., Ohiwa, N., Chang, H., Nishijima, T., Fujikawa, T., Togashi, K., and Saito, T. (2007). Threshold-like pattern of neuronal activation in the hypothalamus during treadmill running: establishment of a minimum running stress (MRS) rat model. Neurosci. Res. 58, 341-348. https://doi.org/10.1016/j.neures.2007.04.004.

29. Messonnier, L.A., Emhoff, C.A., Fattor, J.A., Horning, M.A., Carlson, T.J., and Brooks, G.A. (2013). Lactate kinetics at the lactate threshold in trained and untrained men. J. Appl. Physiol. 114, 1593-1602. https://doi.org/10.1152/japplphysiol.00043.2013.

30. Stapleton, J., Gagnon, D., and Kenny, G.P. (2010). Short-term exercise training does not improve whole-body heat loss when rate of metabolic heat production is considered. Eur. J. Appled. Physiol. 109, 437-446. https://doi.org/10.1007/s00421-010-1380-3.

31. Garber, G.E., Blissmer, B., Deschenes, M.R., Franklin, B.A., Lamonte, M.J., Lee, I.M., Nieman, D.C., Swain, D.P., (2011). American College of Sports Medicine. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med. Sci. Sports. Exerc. 43, 1334-1359. https://doi.org/10.1249/MSS.0b013e318213fefb.

32. Morgan C.J., Renwick, A.G., Friedmann, P.S. (2003). The role of stratum corneum and dermal microvascular perfusion in penetration and tissue levels of water-soluble drugs investigated by microdialysis. Br. J. Dermatol. 148, 434-443. https://doi.org/10.1046/j.1365-2133.2003.05163.x.

33. Morland, C., Andersson, K.A., Haugen, Ø.P., Hadzic, A., Kleppa, L., Gille, A., Rinholm, J.E., Palibrk, V., Diget, E.H., Kennedy, L.H., et al. (2017). Exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1. Nat. Commun. 8, 15557. https://doi.org/10.1038/ncomms15557.

34. Ohno, Y., Nakatani, M., Ito, T., Natsui, Y., Ando, K., Suda, Y., Ohashi, K., Yokoyama, S., and Goto, K. (2023). Activation of lactate receptor positively regulates skeletal muscle mass in mice. Physiol. Res. 72, 465-473. https://doi.org/10.33549/physiolres.935004.

35. Nishikiori, S., Yasuda, J., Murata, K., Takegaki, J., Harada, Y., Shirai, Y., and Fujita, S. (2023). Resistance training rejuvenates aging skin by reducing circulating inflammatory factors and enhancing dermal extracellular matrices. Sci. Rep. 13, 10214. https://doi.org/10.1038/s41598-023-37207-9.

36. Chirumbolo, S., Bertossi, D., and Magistretti, P. (2023). Insights on the role of L-lactate as a signaling molecule in skin aging. Biogerontology 24, 709-726. https://doi.org/10.1007/s10522-023-10018-1.

37. Emholf, C.A.W. and Messonier, L.A. (2023). Concepts of lactate metabolic clearance rate and lactate clamp for metabolic inquiry: a mini-review. Nutrients 15, 3213. https://doi.org/10.3390/nu15143213.

38. Nolt, B., Tu, F., Wang, X., Ha, T., Winter, R., Wiliams, D.L., and Li, C. (2018). Lactate and immunosuppression in sepsis. Shock 49, 120-125. https://doi.org/10.1097/SHK.0000000000000958.

39. Matsui, T., Soya, S., Okamoto, M., Ichitani, Y., Kawanaka, K., and Soya, H. (2011). Brain glycogen decreases during prolonged exercise. J. Physiol. 589, 3383-3393. https://doi.org/10.1113/jphysiol.2010.203570.

40. Matsui, T., Ishikawa, T., Ito, H., Okamoto, M., Inoue, K., Lee, M.C., Fujikawa, T., Ichitani, Y., Kawanaka, K., and Soya H. (2012). Brain glycogen supercompensation following exhaustive exercise. J. Physiol. 590, 607-616. https://doi.org/ 10.1113/jphysiol.2011.217919.

41. Matsui, T., Omuro, H., Liu, Y.F., Soya, M., Shima, T., McEwen, B.S., and Soya, H. (2017). Astrocytic glycogen-derived lactate fuels the brain during exhaustive exercise to maintain

endurance capacity. Proc. Natl. Acad. Sci. U.S.A. 114, 6358-6363. https://doi.org/10.1073/pnas.1702739114.

Figures

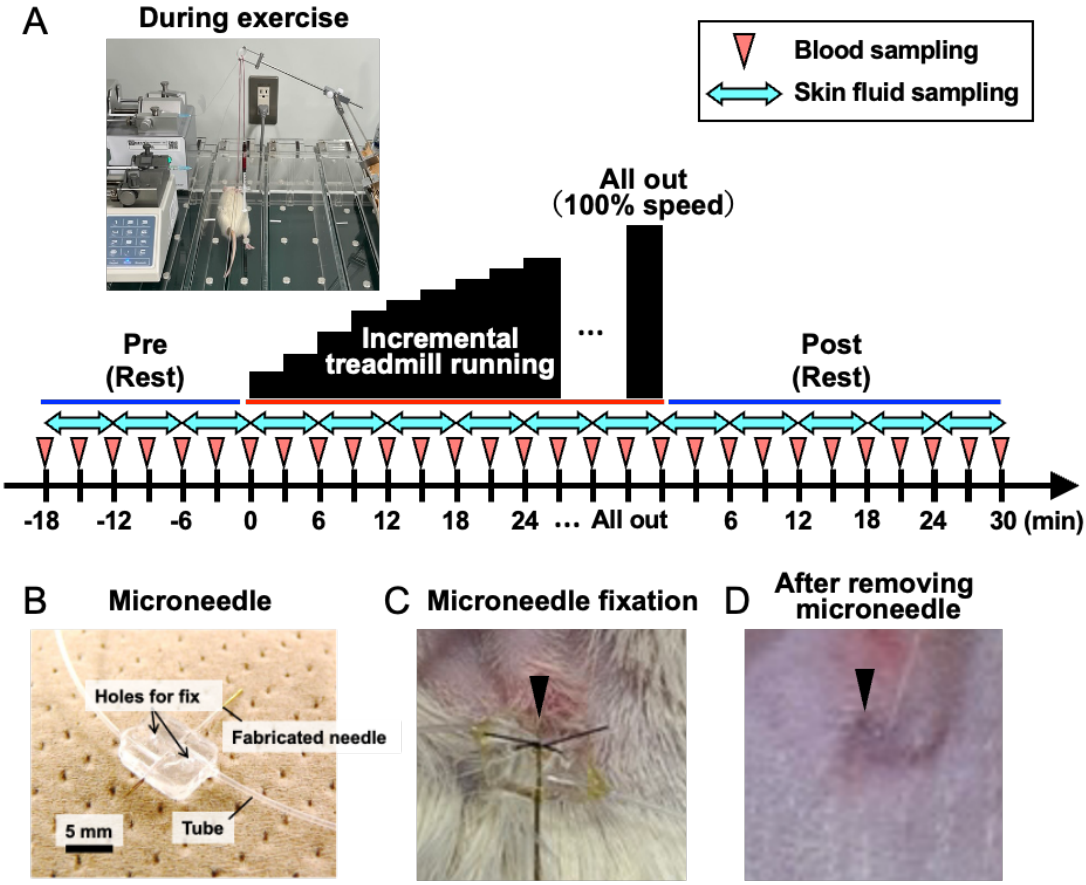


Figure 1. The overview of experimental procedures using a jugular catheter and an ultra-minimally microneedle perfusion system.

(*A*) The experimental settings of rats with inserted catheter and microneedle perfusion system during exercise and experimental protocol of incremental running to determine lactate threshold for rats. (*B*) The specification of microneedle. (*C*) The part of skin with the microneedle inserted and its fixation. (*D*) The part of skin after removing microneedle. (*C* and *D*) indicate no significant inflammation and bleeding, suggesting that the harmful effect of inserting the single microneedle perfusion system may be minimum.

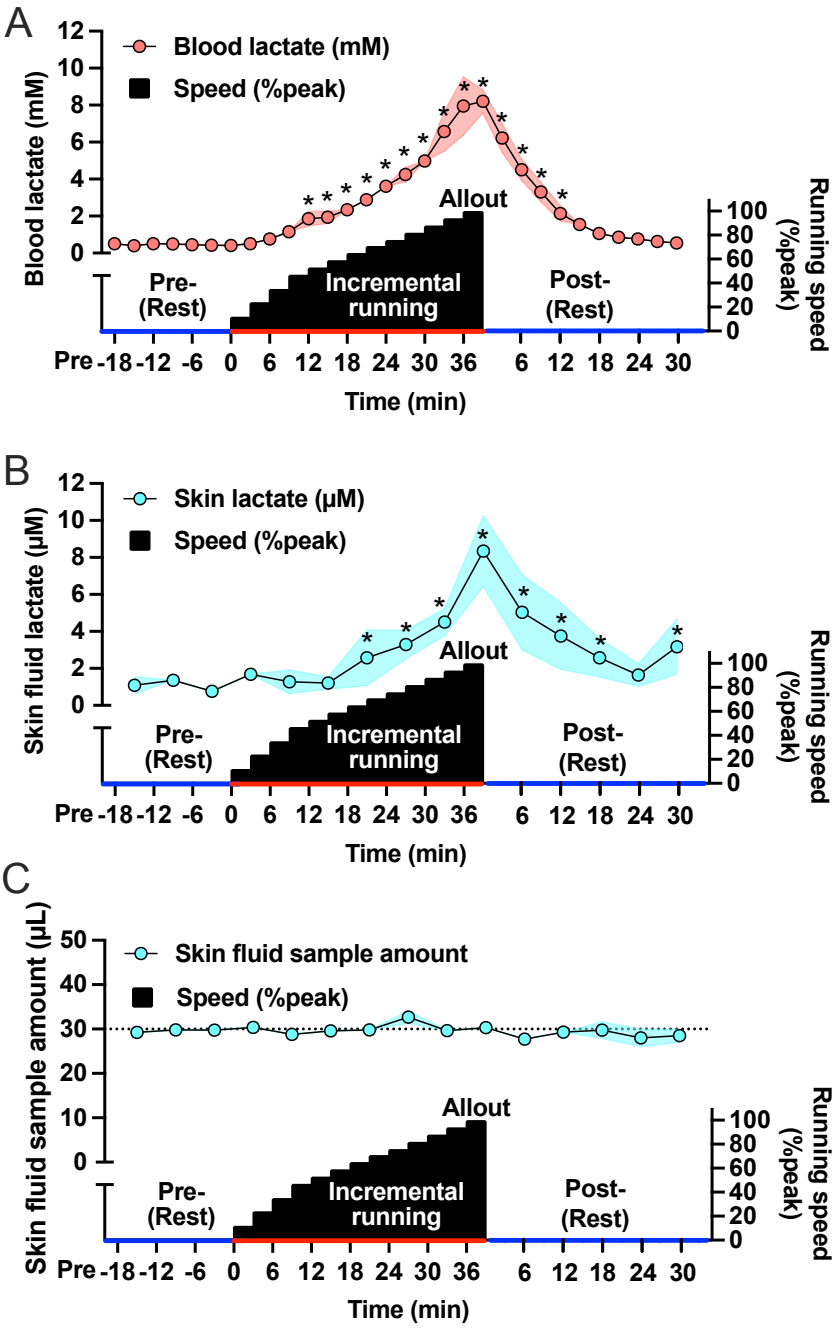


Figure 2. Lactate concentration increases in the skin fluid and blood during incremental treadmill running. Time course changes in blood (*A*) and skin fluid (*B*) lactate concentrations and skin fluid amount (*C*) pre, during, and post incremental running test. Each plot and shaded regions represent mean values and continuous standard error bands, respectively. *$p < 0.05$ vs. 0 min (immediately before the start of the exercise test).

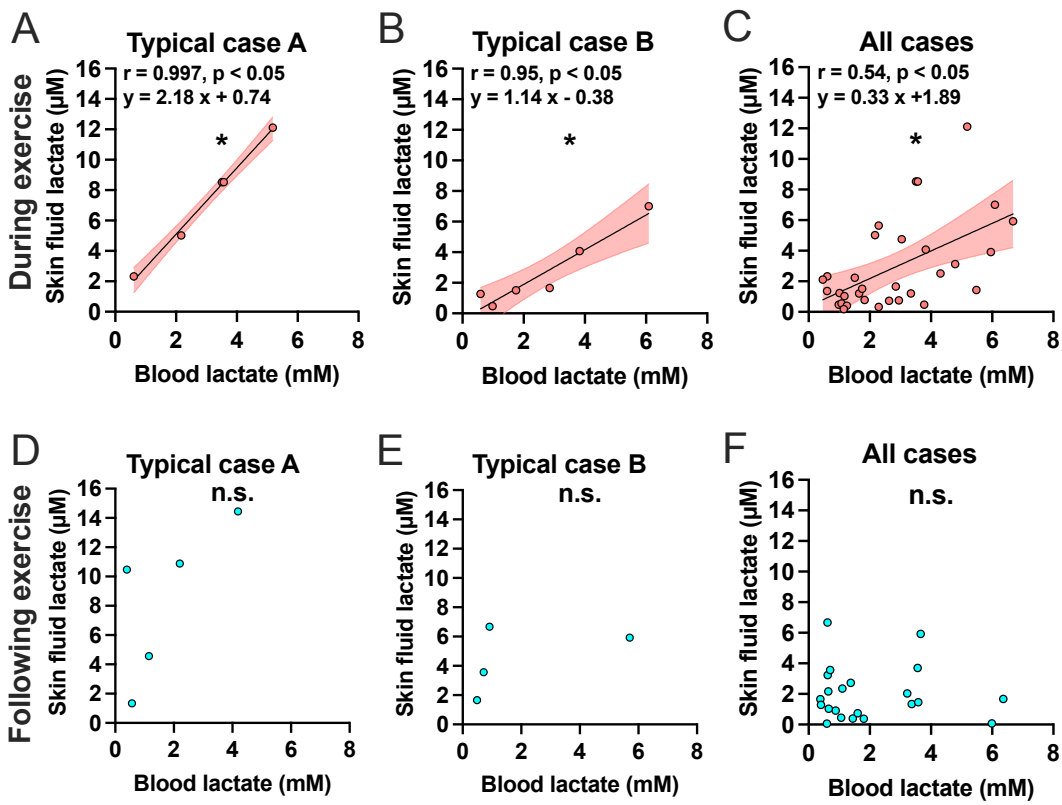


Figure 3. Skin fluid lactate dynamics were significantly associated with blood lactate dynamics during the exercise test but not afterward. The correlation coefficient between the blood and skin fluid lactate concentrations in typical case A and B, and pooled data of all cases during (A-C) and following (*D-F*) exercise. Shaded regions represent 95% simultaneous confidence bands. *p < 0.05, n.s., not significant.

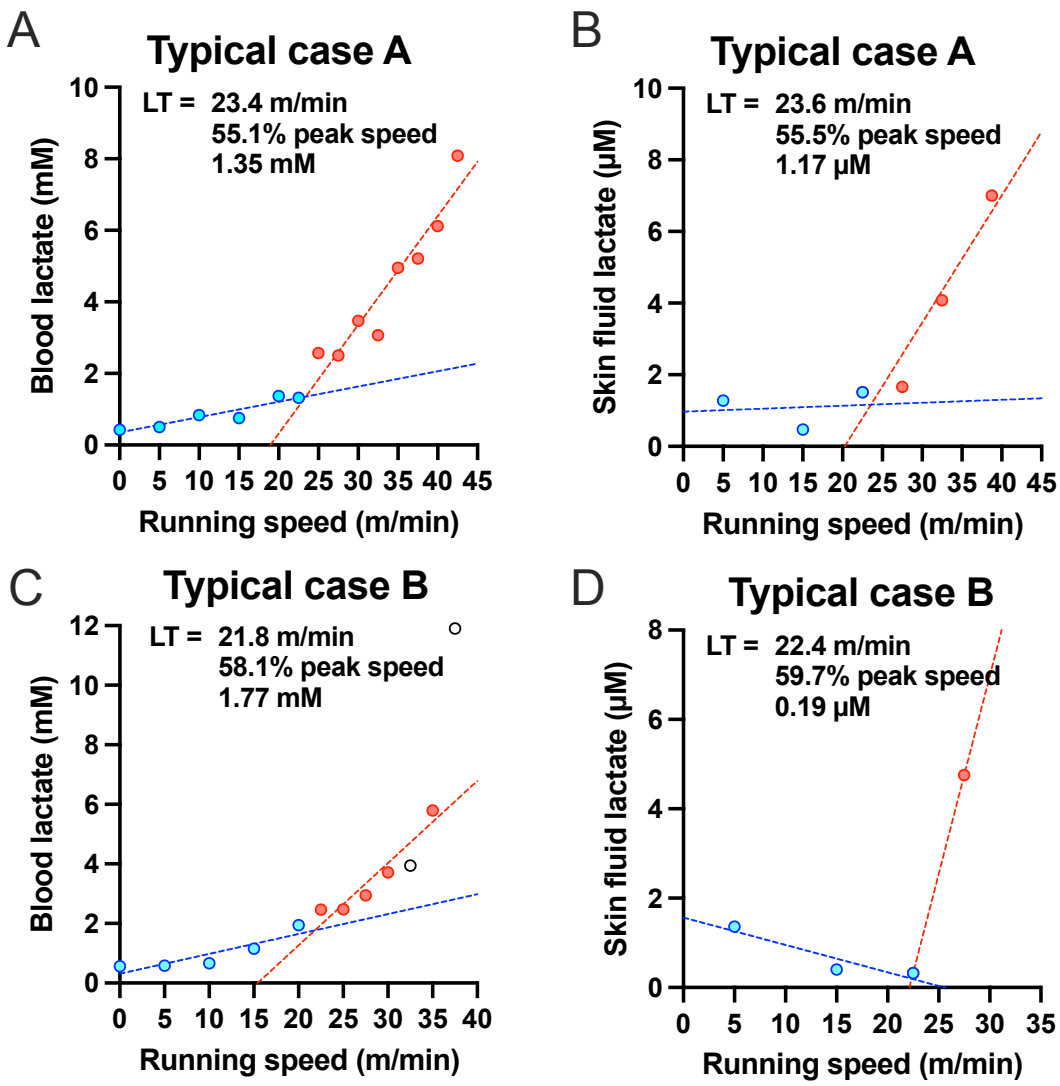


Figure 4. The lactate dynamics in skin fluid can determine lactate threshold as well as blood. Representative LT profiles calculated by the dynamics from blood and skin fluid in typical cases A (panel *A, B*) and B (panel *C, D*). LT in each graph was calculated by *V-slop* method (Beaver et al., 1986).

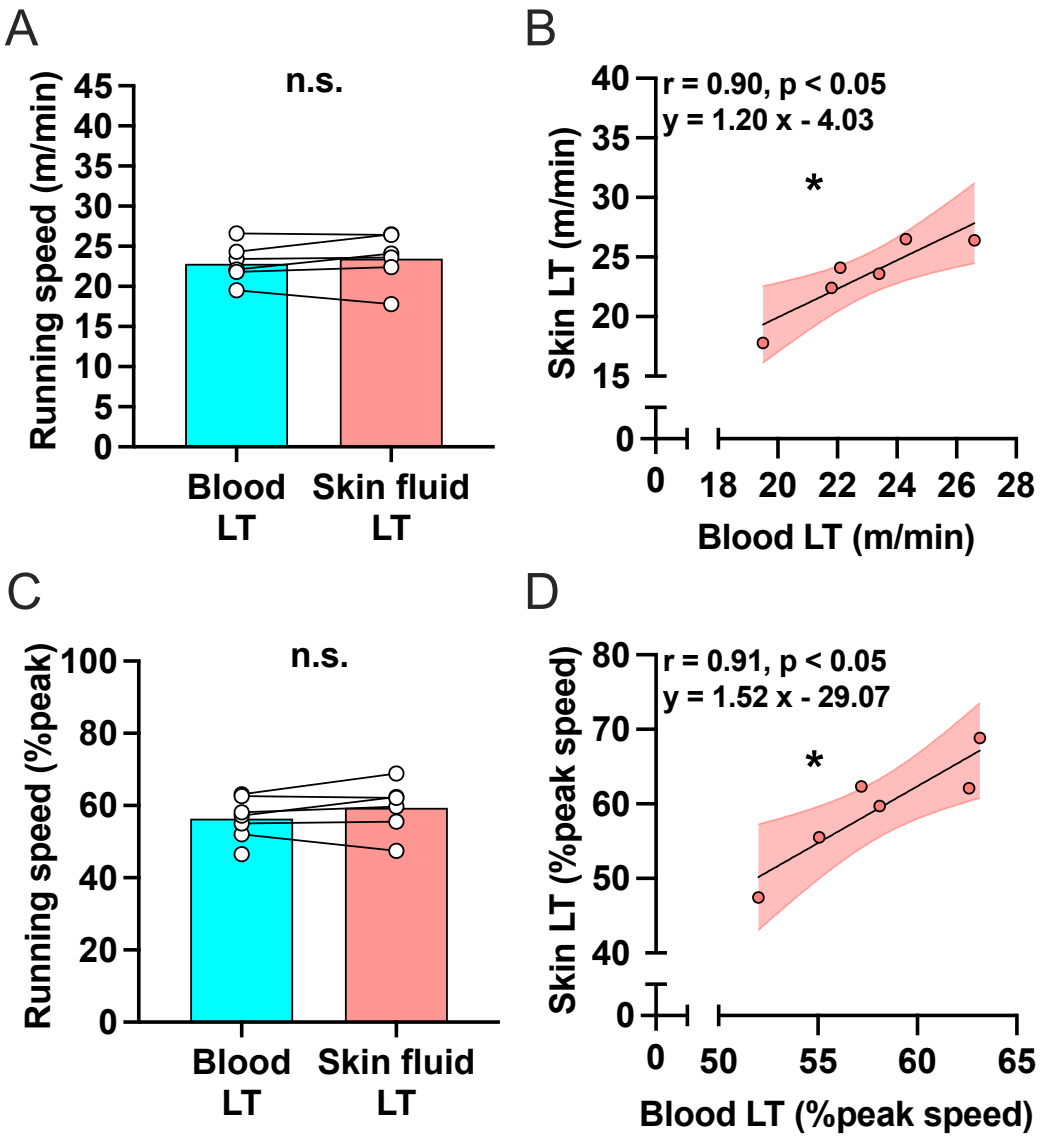
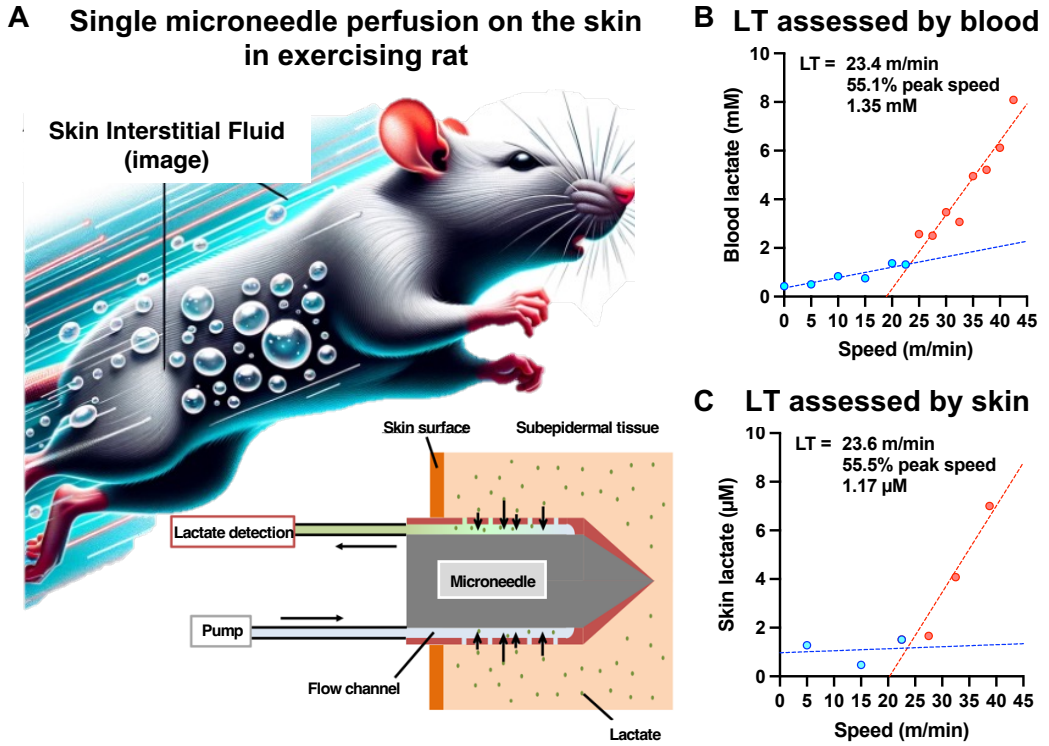
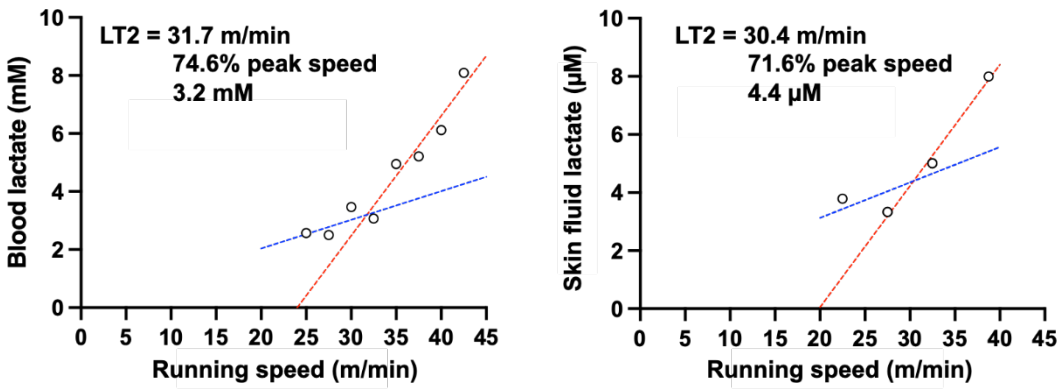


Fig. 5. Lactate threshold calculated from skin fluid lactate are closely associated with those calculated from blood. The absolute running speed corresponding to the LT calculated from blood and skin fluid (A) and their relationship (B). The running speed (C) and its relationship (D) relative to peak speed during incremental exercise corresponding to LT calculated from blood and skin fluid, respectively. The values of bar graph (*A, C*) represent means and individual plots. Shaded regions represent 95% simultaneous confidence bands. n.s., not significant, *$p < 0.05$.



Supplemental Figure 1. Representative graphical image of exercise rats with inserted an ultra-minimally invasive single microneedle (*A*). Representative graph of LT assessed by the blood (*B*) and skin fluid lactate (*C*) dynamics. The findings suggest that LT determination using the perfusate collected by the single microneedle perfusion system can be an effective alternative to current methods for measuring blood lactate, which may be a breakthrough in the development of sports science.



Supplemental Figure 2 Representative case detecting lactate threshold 2 from blood and skin fluid lactate dynamics during the incremental exercise test.