

Simple Lactate Measurement in Horses Using a Portable Lactate Analyzer with Lancet Skin Punctures under Field Conditions

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The following items were investigated to measure blood lactate in horses under field conditions using portable lactate analyzers recently developed for human athletes. 1) The precision of two types of portable lactate analyzer and the standard analyzer using the immobilized enzyme method, and correlation among the lactate concentrations measured by the three lactate analyzers. 2) The correlation between the lactate concentrations in peripheral blood collected by lancet puncture and jugular venous blood using Lactate-pro (L-pro). The study was performed in Thoroughbreds under training for races, using Accusport (AC) and L-pro as portable lactate analyzers and YSI1500 (YSI) for the standard analyzer using the immobilized enzyme method. The intra- and inter-assay coefficients of variation (CV) of L-pro was lower than AC at low and middle lactate concentrations. The precision of L-pro is better than AC when the blood lactate level is lower than 10 mmol/l, and hematocrit (Ht) is 57% or lower. The lactate concentration using L-pro in peripheral blood collected by lancet puncture through the neck skin was similar to the level in jugular venous blood. No infection or inflammatory symptom occurred after blood sampling and the examination is relatively safe. These findings suggest that blood lactate measurement using L-pro by lancet puncture through the neck skin under field conditions is simple and safe, requiring no blood sampling from the jugular vein, and it can be used for evaluation of aerobic capacity and intensity of exercise in daily submaximal training.

Key words: horse, lactate measurement, portable analyzer

J. Equine Sci.
Vol. 18, No. 1
pp. 5–11, 2007

Blood lactate is used for evaluation of aerobic capacity and intensity of exercise for training in humans [1, 8, 21]. However, previous blood lactate analyzers were large, and their calibration was not easy, making measurements difficult under field conditions. Recently, two types of portable lactate analyzer have been developed for human athletes, and a high correlation between the lactate concentrations measured by these analyzers and values measured by the immobilized enzyme method using the current standard blood lactate analyzer has been reported [7, 15]. These analyzers allow measurement of blood lactate using a very small volume of peripheral blood collected from the finger or ear by lancet puncture by athletes themselves, and blood lactate measurement

has become widely performed for scientific management of training.

In horses, blood lactate is considered useful for evaluation of performance, as in humans. In horses, blood lactate has been measured during treadmill tests [14, 18] and running tests under field conditions [5, 9]. It has been reported that the Accusport (AC) portable analyzer should be used only to measure lactate concentration in equine whole blood when Ht is less than 53% and lactate concentration is less than 10 mmol/l [4]. AC requires 15–50 μ l of blood for measurement, and punctured blood has to be collected using a capillary tube to drop on a test strip. Thus, AC use has been limited in horses under field conditions.

The L-pro portable analyzer requires 5 μ l of blood, and lactate can be directly measured through the skin by capillary action. Therefore, it seemed possible that L-pro would be useful for lactate measurement in horses under field conditions. However, there is no

This article was accepted September 26, 2006

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report of measurement of lactate concentration using L-pro in horses, and there are few reports about blood sampling by lancet puncture in horses [11]. Thus, it seemed that there is a problem with the safety of the examination and that of the examiner in lancet puncture.

The following items were investigated for an accurate, safe use of blood lactate measurement in horses under field conditions: 1) the precision of two types of portable lactate analyzer and the standard analyzer using the immobilized enzyme method, and the correlation among the lactate concentrations measured by the three analyzers; and 2) the correlation between the lactate concentrations in peripheral blood collected by lancet puncture and jugular venous blood using L-pro.

Materials and Methods

Experiment 1

Precision of the three lactate analyzers, and correlation among the lactate concentrations measured by the three lactate analyzers

1) Horses

Twenty-eight healthy 2-year-old Thoroughbreds (26 males and 2 females) without abnormality on physical examination before the study were used. All horses were under training for races.

2) Lactate analyzers

Blood lactate was measured by the standard immobilized enzyme method using YSI 1500 Sport (YSI) (Yellow Spring Instrument, Ohio), and two recently developed portable lactate analyzers, Accusport (AC) (Roche Diagnostics, Tokyo) and Lactate-pro (L-Pro) (Arkray, Kyoto). The measurement ranges of YSI, AC and L-Pro were 0.0–30.0 mmol/l, 0.8–22.0 mmol/l, and 0.8–23.3 mmol/l, respectively.

3) Blood sampling and blood lactate measurement

The horses were trained at various intensities of exercise to obtain various lactate concentrations. Blood was collected from the jugular vein after exercise from each horse. Blood was collected in a sodium fluoride/potassium oxalate tube and stored at about 4°C, and blood lactate was measured after 1 hr. Plasma was separated by centrifuging whole blood at 3,000 rpm for 20 min for the examination of the inter-assay CV. All analyzers were calibrated before the test, and

measurements were performed under identical conditions (18°C temperature, 60% humidity).

4) Test items

(1) Intra-assay coefficients of variation

Whole blood samples from horses at three lactate concentrations, low, middle and high were used. They were measured 10 times each by all of the lactate analyzers, and the means, standard deviations (SD) and CV were calculated.

(2) Inter-assay coefficients of variation

Plasma samples from horses at three lactate concentrations, low, middle and high were used. They were measured 3 times daily for 10 days by all of the lactate analyzers, and the means, SD and CV were calculated.

(3) Correlation among the lactate analyzers

Twenty-eight blood samples from each horse were measured 3 times, respectively, using all lactate analyzers, and linear regression analysis and correlation of the means among the 3 lactate analyzers were investigated.

(4) Influence of high lactate level

Since an abnormal value was reported when the blood lactate levels were greater than 10 mmol/l [4], the blood lactate level was divided into lower than 10 mmol/l and 10 mmol/l or higher, and linear regression analysis among the lactate analyzers was performed.

(5) Influence of Ht

Since an abnormal value was reported when Ht was greater than 53% [4], blood was measured three times using the lactate analyzers in each horse, and changes in the SD with an increase in Ht were investigated.

Experiment 2

Correlation between lactate concentrations in peripheral blood collected by lancet puncture and jugular venous blood using L-pro.

1) Horses

Forty-two healthy Thoroughbreds (thirty-four 2-years-old, six 3-years-old and two 4-years-old, 30 males and 12 females) were used. All horses were under training for races.

2) Blood sampling and blood lactate measurement

The horses were trained at various intensities of exercise to obtain various lactate concentrations. The blood samples were collected by puncture using a 21-G lancet (about 7 mm in length) and jugular venous blood 5 min after exercise under field conditions from each horse.

Table 1. Intra-assay coefficients of variation (whole blood sample)

Low concentration (n=10)			
Lactate analyzers	L-pro	AC	YSI
mean (mmol/l)	1.92	2.23	1.99
SD (mmol/l)	0.09	0.12	0.04
CV (%)	4.54	5.32	1.78
Middle concentration			
Lactate analyzers	L-pro	AC	YSI
mean (mmol/l)	8.74	8.97	7.63
SD (mmol/l)	0.28	0.41	0.17
CV (%)	3.20	4.57	2.29
High concentration			
Lactate analyzers	L-pro	AC	YSI
mean (mmol/l)	15.55	13.82	17.50
SD (mmol/l)	0.95	1.10	0.49
CV (%)	6.09	7.98	2.78

Table 2. Inter-assay coefficients of variation (plasma sample)

Low concentration (n=10)			
Lactate analyzers	L-pro	AC	YSI
mean (mmol/l)	2.67	2.22	3.42
SD (mmol/l)	0.09	0.10	0.07
CV (%)	3.37	4.41	2.04
Middle concentration			
Lactate analyzers	L-pro	AC	YSI
mean (mmol/l)	7.50	6.93	6.10
SD (mmol/l)	0.23	0.39	0.13
CV (%)	3.04	5.59	2.11
High concentration			
Lactate analyzers	L-pro	AC	YSI
mean (mmol/l)	12.58	16.51	14.39
SD (mmol/l)	0.46	0.57	0.34
CV (%)	3.62	3.44	2.38

The blood lactate levels were immediately measured using L-pro, and linear regression analysis of the 2 measurements was performed. The puncture site was set at the central region of the left neck for the safety of the blood-sampling person, and hair in an approximately 1-cm² area was shaved before exercise. The blood-samples were collected according to the following procedures. 1) Shaving at the central region of the left neck and disinfection with 50% alcohol to wash off dirt before exercise. 2) Wiping off sweat after exercise and puncture. 3) Wiping off first blood and bleeding again. 4) Measurement of blood lactate. When an insufficient amount of blood was obtained, puncture was repeated. After puncture, the site was disinfected with 50% alcohol. The puncture was observed for infection.

Statistical analysis

Linear regression analysis among the blood lactate concentrations measured using the lactate analyzers was performed. For correlation analysis, Pearson's product-moment correlation coefficient (r) was used. JMP5J (SAS Institute Japan, Tokyo) was used for all tests, and the significance level was set at 5% and 1%.

Results

Experiment 1

1) Intra-assay coefficients of variation

The lowest intra-assay CV was noted in values measured by YSI at all lactate concentrations, followed by L-pro and AC (Table 1). The intra-assay CV was low at the low and middle lactate concentrations for all three lactate analyzers, but the intra-assay CV was high at high lactate concentrations for L-pro and AC.

2) Inter-assay coefficients of variation

As shown in Table 2, the inter-assay CV was low at all lactate concentrations for all three lactate analyzers. The lowest inter-assay CV was noted in values measured by YSI at all lactate concentrations. The inter-assay CV of L-pro was lower than AC at the low and middle lactate concentrations.

3) Linear regression analysis and correlation among the lactate analyzers

The linear regression analysis and coefficient of correlation (r) of the blood lactate levels measured by the three lactate analyzers were: YSI vs. L-pro, $y=0.874x-0.147$ $r=0.96$; YSI vs. AC, $y=0.8x+0.983$, $r=0.93$ and L-pro vs. AC, $y=0.889+1.447x$, $r=0.95$ (Fig. 1). High positive correlations were noted among the three lactate analyzers ($p<0.01$, respectively), and the highest correlation was noted between YSI and L-pro.

4) Influence of high lactate level

High correlations were noted among the three lactate analyzers when the blood lactate levels were

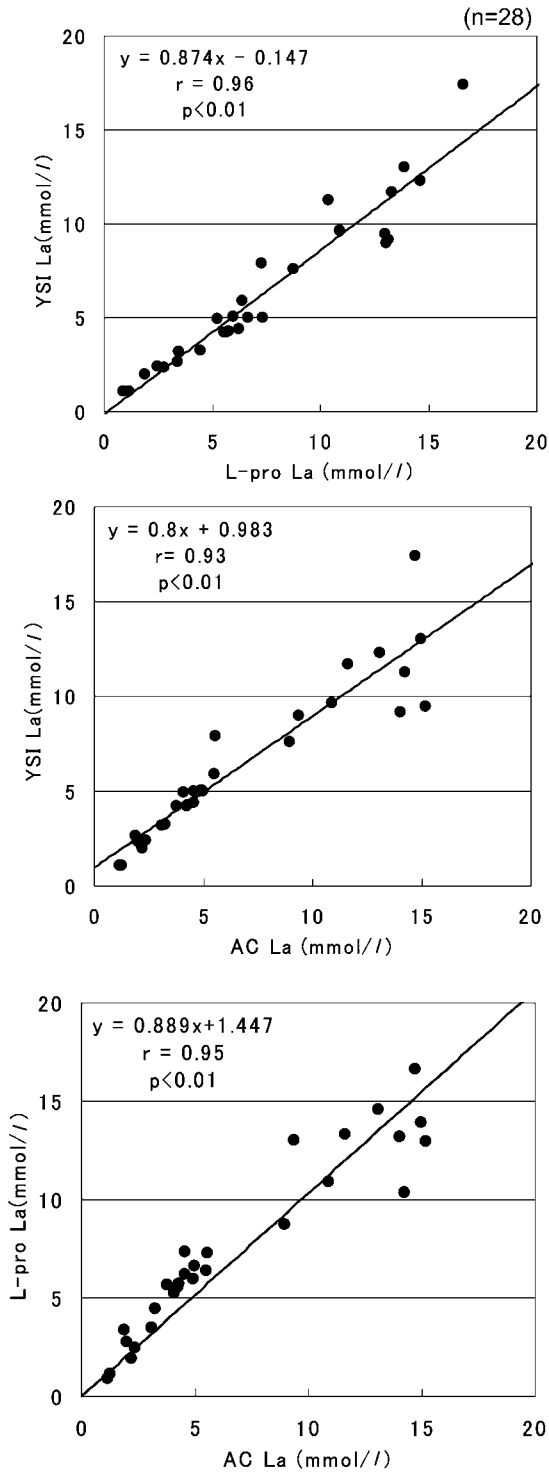


Fig. 1. Linear regression analysis among the blood lactate concentrations by the three lactate analyzers.

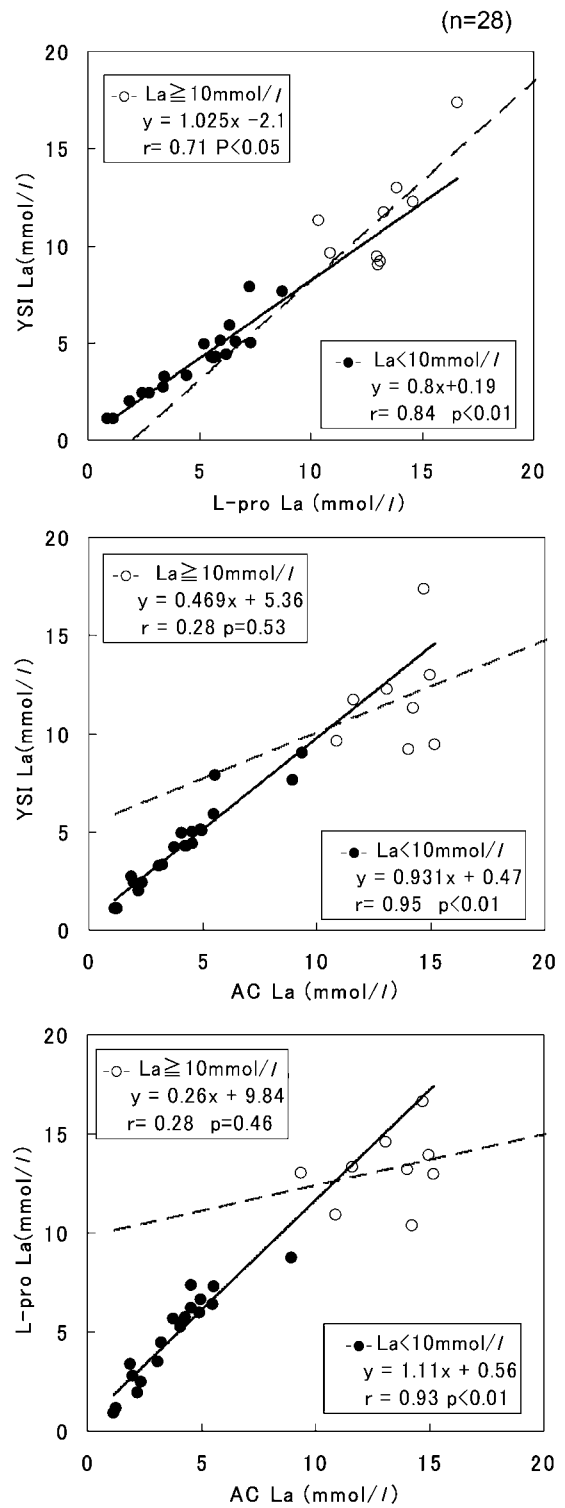


Fig. 2. Change of linear regression analysis among the blood lactate concentrations by the three lactate analyzers (influence of high lactate level).

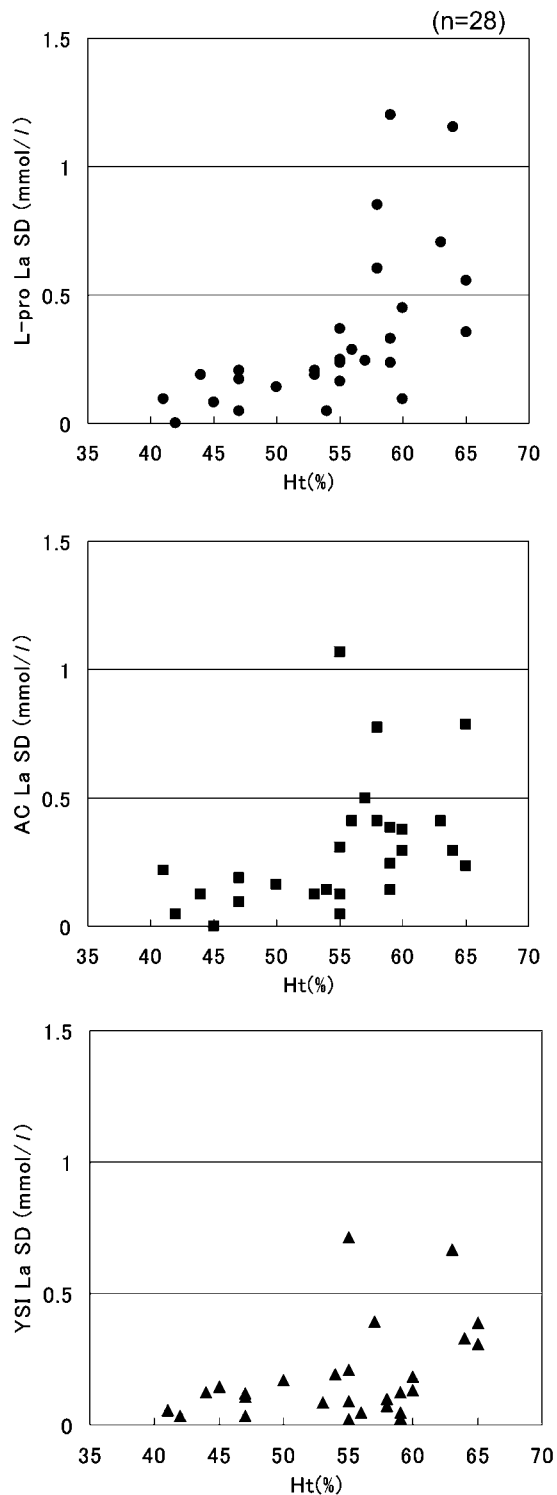


Fig. 3. Change of standard deviation by hematocrit using the three lactate analyzers.

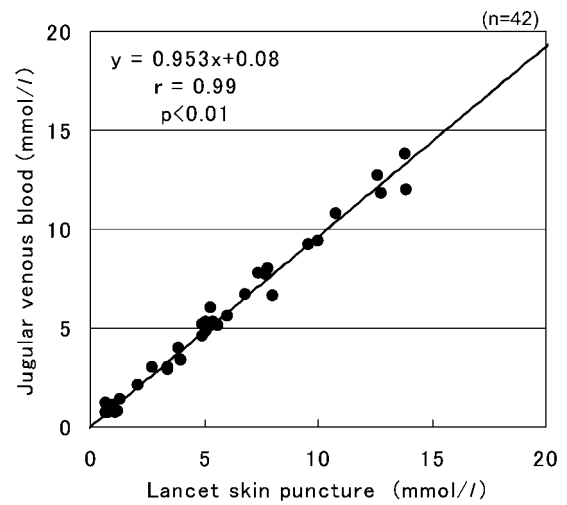


Fig. 4. Linear regression analysis of the blood lactate concentrations by lancet skin puncture and jugular venous blood using L-pro.

lower than 10 mmol/l. However, when the lactate levels were greater than 10 mmol/l, the correlation between YSI and L-pro decreased, and no significant correlation was noted between YSI and AC or between L-pro and AC (Fig. 2).

5) Influence of Ht

When Ht was greater than 54%, the SD of the blood lactate level slightly increased in measurements by YSI and AC. And when Ht was greater than 57%, the SD of the blood lactate level slightly increased in measurements by L-pro (Fig. 3).

Experiment 2

1) Correlation between lactate concentrations in peripheral blood collected by lancet puncture and jugular venous blood using L-pro.

When a sufficient amount of blood was collected using a lancet, a very high correlation ($r=0.99$ $p<0.01$) was noted between lactate concentrations in peripheral blood collected by lancet puncture and jugular venous blood (Fig. 4). No infection was clinically noted in the lancet puncture through the neck skin.

Discussion

The intra-assay CV of measurement using YSI was the lowest among the lactate analyzers, followed by that of L-pro, which was lower than that of AC at all lactate

concentrations. The intra-assay CV increased with the increase in the lactate concentration in measurements using the two portable lactate analyzers. The intra-assay CV was low at the low and middle lactate concentrations. The intra-assay CV of lactate measurement in equine whole blood and plasma using AC has been reported [4, 10, 19], as ranging from 1.6–12.8%. The result of the present study was similar to those of these previous studies.

The inter-assay CV of the three lactate analyzers was low at all concentrations. The lowest inter-assay CV was noted in values measured by YSI at all concentrations. The inter-assay CV of L-pro was lower than that of AC at low and middle lactate concentrations. In reports on the precision of measurements in humans using AC [13] and L-pro [12,16], the inter-assay CV ranged from 2.4–6.4% and 1.1–4.7%, respectively. In this study, the inter-assay CV of the lactate concentrations in equine plasma measured by AC and L-pro were 3.4–5.6% and 3.0–3.6%, respectively. These results show L-pro had a lower inter-assay CV than AC.

The lactate concentrations measured by the two portable analyzers were highly correlated with the values measured by the immobilized enzyme method using YSI. In humans, the correlation coefficients between the lactate concentrations measured by YSI and L-pro and between the values measured by YSI and AC were $r=0.99$ and $r=0.97$, respectively [15]. In horses, the whole blood lactate concentrations calculated with AC correlated with the values measured with the wet chemistry method ($r=0.95$) [10].

In this study, the correlations between the lactate concentrations measured by YSI and L-pro and between the values measured by YSI and AC were high at $r=0.96$ ($p<0.01$) and $r=0.93$ ($P<0.01$), respectively.

It has been reported that the lactate concentrations measured using AC showed abnormal values when Ht was greater than 53% or the blood lactate levels were greater than 10 mmol/l [4]. Thus, the influence of a high lactate level and Ht on the measurement were investigated. Similar to a previous study, when the blood lactate levels were greater than 10 mmol/l, correlation of the lactate concentrations measured by L-pro with those by YSI decreased, and lactate concentration measured by AC lost its correlation with measurements by YSI. Moreover, similar to a previous study, the SD of the blood lactate levels slightly increased in measurements by YSI and AC when Ht was greater than 54%; and when Ht was greater than 57%, the SD of the blood lactate level slightly increased in

measurements by L-pro. Thus, the precision of L-pro was higher than AC. The precision of L-pro may be sufficient when the blood lactate level is lower than 10 mmol/l, and Ht is lower than 57%.

Although it is better to use arterial blood or mixed venous blood for blood lactate measurement, blood sampling is stressful for the examinees. Generally, blood is collected from the fingertip or ear in humans, and from the jugular vein in horses for blood lactate measurement. However, there are many restrictions on blood sampling in horse. Blood sampling should be performed by a veterinarian, and blood sampling from the jugular vein may be difficult due to the horse's nature, making the introduction of blood lactate measurement under field conditions difficult. Thus, we investigated simple blood lactate measurement using lancet skin puncture in horses.

Variation of the blood lactate concentration among blood sampling sites in humans has been reported [2, 3, 6]. A similar finding in horses has recently been reported [17]. Thus, we collected blood from the jugular vein and lancet puncture through the neck skin simultaneously 5 min after exercise under field conditions, and compared the lactate concentrations between the blood samples. In horses, a high correlation between the lactate concentrations in blood collected by lancet puncture of the thoracic skin and collected from the jugular vein has been reported [11]. However, puncture of the thoracic region is less safe and may be difficult due to a harness. We selected the cervical region for lancet puncture because the skin is thin, and in consideration of the safety of the examiner and ease of blood sampling. Since abnormal values were obtained in humans when the blood sample was contaminated with sweat or the amount was small [12], we strictly applied the sampling method described above. A high linearity ($y=0.953x + 0.08$) and very high positive correlation ($r=0.99$) were noted between the lactate concentrations in peripheral blood collected by lancet puncture from the neck skin and jugular venous blood ($p<0.01$). Thus, the lactate concentration in peripheral blood in the neck skin collected by lancet puncture is similar to that in jugular venous blood using L-pro, showing its usefulness for blood lactate measurement.

Blood lactate can be measured under field conditions using L-pro and cervical puncture without blood sampling from the jugular vein. This method is simple and safe, and applicable for evaluation of aerobic capacity and intensity of exercise in daily

training.

Acknowledgments

The author would like to thank the owners and trainers of the horse involved in this study, also Mr. Tomohiro Nagahara for his help in the collection of the data, and Dr. Atsushi Hiraga for his advice. In addition, the author would also like to special thank Dr. Akio Amada for his help and support in performing this study.

References

- Allen, W.K., Seals, D.R., Hurley, B.F., Ehsani, A.A., and Hagberg, J.M. 1985. Lactate threshold and distance-running performance in young and older endurance athletes. *J. Appl. Physiol.* **58**: 1281–1284.
- Dassonville, J., Beillot, J., Lessard, Y., Jan, J., Andre, A.M., Le Pourcelet, C., Rochcongar, P., and Carre, F. 1998. Blood lactate concentrations during exercise: effect of sampling site and exercise mode. *J. Sports Med. Phys. Fitness* **38**: 39–46.
- el-Sayed, M.S., George, K.P., and Dyson, K. 1993. The influence of blood sampling site on lactate concentration during submaximal exercise at 4 mmol.l⁻¹ lactate level. *Eur. J. Appl. Physiol. Occup. Physiol.* **67**: 518–522.
- Evans, D.L., Harris, R.C., and Snow, D.H. 1993. Correlation of racing performance with blood lactate and heart rate after exercise in thoroughbred horses. *Equine Vet. J.* **25**: 441–445.
- Evans, D.L., and Golland, L.C. 1996. Accuracy of Accusport for measurement of lactate concentrations in equine blood and plasma. *Equine Vet. J.* **28**: 398–402.
- Feliu, J., Ventura, J.L., Segura, R., Rodas, G., Riera, J., Estruch, A., Zamora, A., and Capdevila, L. 1999. Differences between lactate concentration of samples from ear lobe and the fingertip. *J. Physiol. Biochem.* **55**: 333–339.
- Fell, J.W., Rayfield, J.M., Gulbin, J.P., and Gaffney, P.T. 1998. Evaluation of the Accusport Lactate Analyzer. *Int. J. Sports Med.* **19**: 199–204.
- Hagberg, J.M., and Coyle, E.F. 1983. Physiological determinants of endurance performance as studied in competitive racewalkers. *Med. Sci. Sports Exerc.* **15**: 287–289.
- Harkins, J.D., Beadle, R.E., and Kamerling, S.G. 1993. The correlation of running ability and physiological variables in Thoroughbred racehorses. *Equine Vet. J.* **25**: 53–60.
- Lindner, A. 1996. Measurement of plasma lactate concentration with Accusport. *Equine Vet. J.* **28**: 403–405.
- Lindner, A., and Birks, E.K. 1994. Collection of venous blood samples from competition horses: a new approach. *Equine Vet. J.* **26**: 503–505.
- Makita, S., and Satomi, J. 1997. The reliability of the portable lactate analyzer (Lactate-pro). *Clinical Sports Medicine* **14**: 815–819.
- Miyamoto, Y., Yamauchi, M., Shindo, M., and Tanaka, H. 1996. Precision of the portable lactate analyzer (Accusport). *Clinical Sports Medicine* **13**: 537–541.
- Persson, S.G. 1997. Heart rate and blood lactate responses to submaximal treadmill exercise in the normally performing standardbred trotter—age and sex variations and predictability from the total red blood cell volume. *Zentralbl Veterinarmed A.* **44**: 125–132.
- Pyne, D.B., Boston, T., Martin, D.T., and Logan, A. 2000. Evaluation of the Lactate Pro blood lactate analyzer. *Eur. J. Appl. Physiol.* **82**: 112–116.
- Saiga, K., Komatsu, Y., Yamanaka, S., Nishimori, Y., Shimamaki, M., Niigaki, N., Nishimori, M., and Sasaki, T. 1997. Evaluation of the portable lactate analyzer (Lactate Pro). *Clinical Examination Equipment and Reagent* **20**: 535–539.
- Schott, H.C. 2nd., Bohart, G.V., and Eberhart, S.W. 2002. Potassium and lactate uptake by noncontracting tissue during strenuous exercise. *Equine Vet. J. Suppl.* **34**: 532–538.
- Seeherman, H.J., and Morris, E.A. 1990. Methodology and repeatability of a standardized treadmill exercise test for clinical evaluation of fitness in horses. *Equine Vet. J. Suppl.* **9**: 20–25.
- Williamson, C.C., James, E.A., James, M.P., May, C.D., and Casey, P.J. 1996. Horse plasma lactate determinations: comparison of wet and dry chemistry methods and the effect of storage. *Equine Vet. J.* **28**: 406–408.
- Yoshida, T. 1984. Effect of exercise duration during incremental exercise on the determination of anaerobic threshold and the onset of blood lactate accumulation. *Eur. J. Appl. Physiol. Occup. Physiol.* **53**: 196–199.
- Yoshida, T., Chida, M., Ichioka, M., and Suda, Y. 1987. Blood lactate parameters related to aerobic capacity and endurance performance. *Eur. J. Appl. Physiol. Occup. Physiol.* **56**: 7–11.