Evaluation of the Lactate Threshold of Rats Using External Jugular Vein Catheterization

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Abstract. [Purpose] The purpose of the present study was to investigate if lactate thresholds in rats could be accurately determined from sequential blood lactate concentrations during incremental exercise tests. [Subjects] Six female Wistar rats were used in this study. [Methods] Catheters were inserted into the right external jugular veins of the rats before they performed the incremental exercise tests. To determine the lactate threshold, a graph was made from the results of the blood lactate concentrations at the different running speeds. [Results] It was possible to frequently and reliably measure blood lactate concentrations of the rats during incremental exercise tests and to determine lactate thresholds. The mean lactate concentration at the lactate threshold was 3.5 ±3.0 mmol/L, and the mean running speed was 18.0 ± 2.8 (16.1–22.8) m/min. [Conclusion] This study showed that it is possible to accurately determine the lactate threshold by taking sequential blood samples during incremental exercise tests.

Key words: Lactate threshold, External jugular vein catheterization, Incremental exercise test

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INTRODUCTION

Evaluation of exercise capacity is a very important indicator of exercise prescription in sports medicine1) and rehabilitation2). There are some reports showing that maximal oxygen uptake3, 4) and lactate threshold5–8) can be used to determine the exercise capacity of rats. However, measurement of maximal oxygen uptake needs special equipment, and it is not possible to measure the ventilation response and anaerobic threshold during treadmill running when using a mixing chamber.

To determine the lactate threshold, it is necessary to take sequential blood samples during incremental exercise tests. Use of the tail vein for blood sampling5, 6) cannot be used when performing incremental exercise tests, because the tail must be stabilized to draw blood, which prevents the rat from running. It is possible to take blood lactate concentrations frequently when using an external jugular vein catheterization. However, previous attempts7, 8) were not successful at taking enough blood to accurately determine the lactate threshold during incremental exercise tests, and arbitrary analyses were used, which led to unreliable results.

In this study, we propose a protocol for the accurate determination of the lactate threshold using external jugular vein catheterization during incremental exercise tests.

SUBJECTS AND METHODS

Six female Wistar rats (12 weeks of age, 276.7 ±18.6 g) were used in this study. The animals were housed in a temperature-controlled room at 23 °C with a 12-hour light-dark cycle, and were given free access to standard rat food and water. The protocols of this study were approved by the Animal Experiment Committee of the Prefectural University of Hiroshima (No. M11-0023).

All rats were trained to do treadmill running (15 m/min, 5° inclination, for 10 min/day) for six days. A treadmill made for rats and mice (Exer-3/6, Columbus Co., Ltd) was used.

After this training period, the rats were anesthetized with diethyl ether, and a catheter was inserted into the right external jugular vein for blood sampling according to method reported by Hashimoto et al9).

Heparinized saline (100 IU/ml) was infused into the catheter to prevent blood coagulation.

Two days after the operation, the rats performed incremental exercise tests. The exercise test continued until the rat could no longer run. The initial speed of the treadmill was 10 m/min, and the treadmill speed was increased every 2 minutes by 2 m/min. Blood samples (5 µL) were taken from the jugular vein catheter at rest and every 2 minutes after-
wards for the analysis. Blood lactate concentrations were measured using a portable lactate analyzer (Lactate Pro; Arkray, Japan). The lactate analyzer software (MEQNET LT Manager; Arkray, Japan) determined the 2-line combination that minimizes the total sum of squares for fitting all data\(^6\).

To determine the lactate threshold, a graph was made from the results of the blood lactate concentrations at the different running speeds. The lactate threshold was considered to lie at the intersection of the 2 lines\(^{10}\).

**RESULTS**

The mean blood lactate concentration was 2.6 ± 0.6 mmol/L at rest. The maximal lactate concentration was 13.1 ± 4.4 mmol/L during the exercise tests. The mean lactate concentration at the lactate threshold was 3.5 ± 3.0 mmol/L, and the mean running speed was 18.0 ± 2.8 (16.1–22.8) m/min (Table 1). The lactate threshold for rat number 3 could not be determined because the data were too variable.

**DISCUSSION**

The evaluation of the lactate threshold in healthy humans\(^{11, 12}\) is done by taking frequent blood samples every 1 to 3 minutes, during incremental exercise tests. In this study, the authors were able to accurately determine the lactate threshold of rats by taking sequential blood samples during incremental exercise tests, as is done in human testing.

Makita et al.\(^{13}\) demonstrated that a portable lactate analyzer has sufficient reliability and validity, because tests of various blood samples (e.g. blood density, volume and contaminants, etc.) had sufficient accuracy and were repeatable; in addition, blood volume tests and correlation tests could be performed. The software they used made it possible to easily determine the lactate threshold, as clear lines for the blood lactate concentrations versus running speeds were drawn automatically.

Takahashi et al.\(^6\) found the running speed of rats at the lactate threshold was 20 m/min, and the blood lactate concentration at the lactate threshold was 1.4 mmol/L. The results obtained by Takahashi et al.\(^6\) were higher than those we report in the present study. The results of Takahashi et al.\(^6\) were obtained by drawing blood from the tail vein during the rest period, not while the rat was running. Using our method, it was possible to frequently and reliably measure blood lactate concentrations during the incremental exercise tests for all the rats.

Soya et al.\(^7\) used external jugular vein catheterization to estimate the lactate threshold of rats. They reported the running speed of rats at the lactate threshold was 10–20 m/min, but they did not report the lactate concentration at the lactate threshold. The most likely reason Soya et al.\(^7\) were not able to draw a regression line was because the blood lactate concentrations were not measured frequently enough as the treadmill speed was increased every 4 minutes by 5 m/min. The protocol of the present study was adequate for determining the lactate threshold, because the treadmill speed was increased every 2 minutes by 2 m/min. It was possible to draw a clear inflection point in the curve of lactate concentration versus running speed.

In the present study, the running speed at the lactate

### Table 1. Changes in blood lactate concentration during exercise: blood lactate concentration and treadmill speed at lactate threshold

<table>
<thead>
<tr>
<th>Time (m)</th>
<th>Treadmill Speed (m/min)</th>
<th>Blood Lactate (mmol/L)</th>
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<tr>
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</table>

LT: Lactate threshold
threshold varied from 16.1 to 22.8 m/min, a range of up to 6.7 m/min. Furthermore, the lactate concentrations at the lactate threshold varied from 1.2 to 8.7 mmol/L. However, the concentrations of the lactate in each rat did not vary during the first half of running. Our results were similar to the patterns observed in normal humans\(^\text{12}\). Therefore, even in same-age rats, the aerobic capacity might be subject to individual differences. Consequently, our present results suggest that it is necessary to measure individual lactate thresholds before performing experiments using exercise to determine the aerobic capacity of rats.

The authors believe that the results of the current research provide important basic data for experiments using exercise involving rats, because our results are more reliable and the blood samples were drawn while the rats continued running.

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