Indian Journal of Experimental Biology
Vol. 49, October 2011, pp. 781-785

Non-exhaustive test for aerobic capacity determination in running rats

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Received 24 February 2011; revised 27 June 2011

A simple and applicable method for non-exhaustive aerobic evaluation in running rats is described. Wistar rats were submitted to running test at different velocities (10, 15, 20, 25 m/min) with 48 h recovery among them. At each velocity, the rats ran two bouts of 5 min with 2 min of rest between bouts. Blood samples were collected at the end of each bout for lactate determination. For each intensity, delta lactate was calculated and using deltas obtained by four tests, an individual linear interpolation was plotted. The y-intercept of linear interpolation was the "null delta lactate" equivalent to the critical velocity (CV). To verify the lactate stabilization at CV, the animals were submitted to 25 min of continuous exercise (15, 20, 25 m/min), with blood collection every 5 min. The estimated CV was 16.6±0.7 m/min, with significant linear regressions (R=0.90±0.03). The rats presented maximal lactate steady state (MLSS) at 3.9±0.4 mmol/L, at 20 m/min. The CV was less than MLSS but significantly correlated with this parameter (r=0.78). This non-exhaustive test seems to be valid for the aerobic evaluation of sedentary rats and this protocol underestimates the MLSS in 20%. This test seems to be the interesting method for the evaluation of rats submitted to acute exercise or physical training.

Keywords: Blood lactate, Critical load, Double bouts exercise test, Maximal lactate steady state, Running

The aerobic/anaerobic transition zone showed a great importance for evaluation and exercise training prescription. For this reason, in the last decades, numerous investigations have produced different protocols for identifying this metabolic transition.

The anaerobic threshold (AT) is defined as the workload at which the blood lactate starts to accumulate excessively during progressive exercises. In human beings, AT is usually determined by submitting the individual to efforts with loads progressively higher with concomitant evaluation of blood lactate concentration. Theoretically, AT indicates the workload equivalent to the maximal lactate steady state (MLSS); in other words, the highest exercise intensity at which blood lactate does not increase beyond the initial transient during a constant load exercise[1,2].

A double bouts test for non-exhaustive aerobic capacity determination in human beings was purposed by Chassain[3]. This method was based on blood lactate, heart rate (HR) and oxygen uptake (VO_2) responses to exercise. This procedure assumed that the aerobic-anaerobic transition is demarcated by the highest exercise intensity at which physiological variables do not increase beyond the initial transient increase during a constant load exercise, i.e., a maximal blood lactate, HR and VO_2 steady state. This method consists of having participants perform two exercise bouts of equal intensity for 3 min, with passive recovery of 1.5 min between subsequent exercise bouts[3]. For each exercise intensity, a value of delta blood lactate, HR and VO_2 is calculated by subtracting the blood lactate concentration, HR and VO_2 at the end of the first session from the blood lactate concentration, HR and VO_2 at the end of the second session. An individual interpolation is plotted with the delta lactate, HR and VO_2 for each load, allowing, the calculation of a "null exercise intensity", i.e., the highest intensity at which there is no significant blood lactate, HR and VO_2 increase. The intensity corresponding to the "null variation" for each physiological variable indicates, at least theoretically, the maximal steady state for blood lactate, HR and VO_2 and was named "critical power".

Since there are limitations in the studies with human beings, animal model provide conditions for attainment of information which would not be possible in the exercise physiology and health science.

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There is a great interest in the estimation of the intensity of the effort performed by rats during exercise, due to the use of these animals in several investigation areas. With the exercise intensity determination in animals, the transference of knowledge for studies with human beings is possible. The physical tests applied to rats can supply information concerning the beneficial or deleterious metabolic alterations promoted by diseases in relation to homeostasis preservation and physical training capacity. Besides, animal exercise models allow invasive procedures to be accomplished in a way to improve the understanding of cellular and molecular mechanisms related to the exercise.

Manchado *et al*.[4] conducted a study aimed to adapt the Chassain´s protocol for swimming rats aerobic evaluation. The reliability of this method for aerobic evaluation and its relation with the maximal lactate steady state in rats during swimming exercise was demonstrated.

Treadmill running also is an important exercise type for the evaluation of metabolism in rats[5-7]. Pillis *et al*.[8] analyzed the blood lactate concentration in rats during a multistage treadmill exercise and calculated the anaerobic threshold (AT). In this study, the blood lactate showed a pattern similar to that described in human beings and the AT was observed at a velocity of 25 m/min, with a blood lactate concentration of 4.0 mmol/L.

Manchado *et al*.[9] determined the maximal lactate steady state of running rats, using the continuous running intensity and blood colleted each 5 minutes of effort. The velocity associated to MLSS in running rats was 20 m/min and the blood lactate concentration stabilized at 3.9 mmol/L. Similar results were described by Contarteze *et al*.[10]. Billat *et al*.[11] also checked the mouse critical running speed using a non-invasive but exhaustive procedure and Manchado-Gobatto *et al*.[12] adopted the similar protocol to evaluate running Wistar rats. Despite the importance of these experiments, most protocols applied in running rodents use exhaustive procedures. This is a great limitation of these protocols especially for exercise prescription of animals with pathologies and elderly.

In this context, the aim of the present study is to describe a double bouts exercise method for non-exhaustive aerobic conditioning determination in running rats, based on the protocol originally described by Chassain[3], for human beings and standardization for swimming rats.

Materials and Methods

Animals—Thirteen untrained male Wistar rats (*Rattus norvegicus*), 120 days old, weighing 310-390 g at the beginning and 410-480 g at the end of the experiment, were used. During the whole experiment, the animals received water and commercial chow Labina-Purina *ad libitum*. The rats were housed in collective cages (5 animals per cage), in a room with turned on lights from 06.00 to 18.00 hrs, at a 25°C. All experiments involving animals were conducted in accordance with the policy statement of the American College of Sports Medicine on Research with experimental animals.

Selection of running rats and adaptation to the treadmill exercise—The process of selection of running rats took place for ten consecutive days, with the animals running to 5 min at 15 m/min every day. The animals that got to run nine or ten times were selected. Later, the animals were adapted to the treadmill. The adaptation consisted of keeping the rats to run for three weeks, 5 days/week, with duration and speed increased progressively. The purpose of the adaptation was to reduce potential exercise stress without promoting physical training adaptations[12].

Exercise-test protocol

Double bouts exercise method—The rats were individually submitted to four running tests at different velocities (10, 15, 20 and 25 m/min) executed at random, and with intervals of 48 h among them. These velocities were selected based on a previous study where it was observed that the MLSS intensity corresponded to 20 m/min[9-10]. Therefore, velocities below and above of the MLSS intensity were selected. The non-exhaustive method is characterized by double bouts exercise, at the same intensity, separated by a passive recovery. In the present study, the rats run twice for 5 min each time, at same intensity, with a 2 min interval between the two exercise bouts. Blood samples were collected at the end of each bout for lactate determination. For each intensity the value of delta lactate was calculated by subtracting the blood lactate concentration at the end of the first bout (LAC_{E1}) from the lactate concentration at the end of the second bout (LAC_{E2}). With the delta lactate for each load, an individual linear interpolation was plotted, which enabled the calculation of a "null lactate delta", equivalent to the critical velocity (CV).

Determination of the maximal lactate steady state— To check the possible stabilization of the blood lactate at the CV intensity obtained by double exercise methods, the rats were submitted to 25 min of continuous exercise at intensities equivalent to 15, 20 and 25 m/min, also at random, and with intervals of 48 h among them. The highest intensity at which the increase on the blood lactate concentration was equal to or below 1 mmol/L from the 10th to the 25th min was considered as the maximal lactate steady state[9,13].

Blood samples and analysis—Blood samples (25 µl) were collected from a cut at the tail tip during the exercise tests and deposited in *Eppendorf* tubes (1.5 ml capacity) containing 50µl sodium fluoride (1%). To avoid blood lactate dilution with residual water at the tail of the animal, the rats were quickly dried with a towel, immediately before blood collection. The lactate concentrations were determined in lactate analyzer (YSI model 1500 SPORT).

Statistical analysis—The statistical procedure consisted of one-way ANOVA. When necessary, the Newman-Keuls post-hoc comparison test was used[14]. The Pearson Correlation test identifies the correlation between critical velocity and maximal lactate steady state. The statistical package used was STATISTICA 7.0 (StatSoft, Inc., Tulsa, OK). In all cases, the statistical significance was set at $P < 0.05$.

Results

There was a linear increase in delta blood lactate according to the increase in effort intensity. The estimated critical velocity was 16.6 ± 0.7 m/min, with significant linear regressions (R=0.90 ± 0.03) (Table 1).

The animals presented maximal steady state at 3.90 ± 0.35 mmol/L of blood lactate, at the 20 m/min intensity. At 15 m/min, there was also stabilization of blood lactate, but in lower concentration (3.05±0.34 mmol/L). There was a progressive increase in blood lactate concentration at 25 m/min, and, some animals reached exhaustion between the 10th and 25th min of exercise (Fig. 1).

Discussion

The exercise physiology often uses experimental animal models to observe the physiological responses to exercise, especially for invasive manipulations and pathology studies, but few researches are designed to evaluate and quantify intensity of the effort performed by animals during exercise[8,9,11-13,15,16]. Therefore, it is necessary to develop simple methods for physical evaluation of exercised rats.

The present study describes an adaptation of the non-exhaustive protocol for critical power originally suggested for human beings[3], to aerobic evaluation of running rats[4].

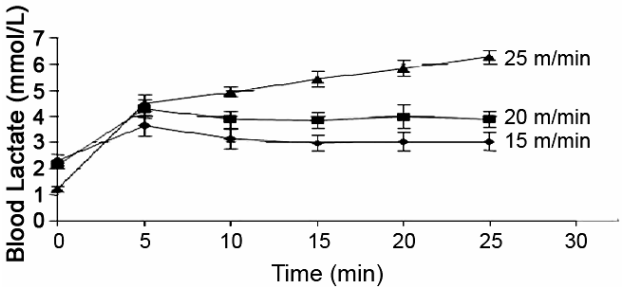

Fig. 1— Blood lactate concentrations during exercise tests in intensities corresponding to 15, 20 and 25 m/min [Values are mean ± SE from 13 observations each.

Table 1—Delta lactate for each velocity (10, 15, 20 and 25 m/min), estimated individual critical velocity (CV) and linear regression coefficient (R) value of rats

Rat	10 m/min (mmol/L)	15 m/min (mmol/L)	20 m/min (mmol/L)	25 m/min (mmol/L)	CV (m/min)	R
1	- 0.21	- 0.06	- 0.06	0.21	18.53	0.93
2	- 0.69	- 0.27	0.33	0.60	17.58	0.99
3	- 0.72	- 0.03	0.93	1.23	15.00	0.98
4	- 0.54	1.05	0.63	1.08	13.92	0.75
5	- 0.99	- 0.48	- 0.24	1.62	17.62	0.92
6	- 0.82	- 0.72	0.21	0.23	20.35	0.92
7	- 0.66	- 0.35	0.06	0.48	19.03	1.00
8	- 0.18	0.33	0.63	0.90	11.72	0.98
9	- 0.15	- 0.51	- 0.44	0.66	18.30	0.60
10	- 0.60	- 0.09	- 0.17	0.67	18.03	0.91
11	- 0.40	0.45	0.51	1.20	13.35	0.96
12	- 0.70	- 0.66	- 0.67	1.82	17.71	0.78
13	- 0.06	- 0.01	0.24	1.49	14.31	0.92
Mean	- 0.52	- 0.10	0.15	0.94	16.57	0.90
SEM	0.08	0.14	0.13	0.14	0.72	0.03

All the rats showed acceptable and linear values of delta lactate, increasing progressively with the increase of the double effort intensity (Table 1). The same results were observed in double bouts protocol adapted for swimming rats[4] and in the original method described by Chassain[3], executed with young human subjects exercised in a cycle ergometer.

The double bout effort-pause relation adopted in the present study was similar to that applied to swimming rats[4] but different from that of Chassain[3], who used two exercises session of equal intensity, lasting 3 min with 1.5 min recovery in between the exercise bouts. Protocols for evaluation of swimming and running rats usually employ 5 min exercise stages to elevate blood lactate above the base value[9,16]. For this reason, 5 min double bouts of effort separated by a passive recovery of 2 min, resulting in an effort-pause relation similar to that by Chassain[4] was used in the present study.

The major advantage of double bouts test applied for rats is the non-exhaustive characteristic of this procedure. Most protocols for aerobic and anaerobic evaluation of animals, during physical exercise are exhaustive[8,11,15-17], resulting in restrictions for practical applications of the tests, in especially in studies using animal models of pathologies, drugs and aging.

The maximal lactate steady state during continuous exercise was used to validate the present protocol since it corresponds to the highest blood lactate concentration (BLC) at which lactate removal capacity compensates its entry[18-20] and is considered the "gold standard" method for determination of aerobic-anaerobic metabolism transition[2,13,18].

In the present study, the MLSS was obtained at the intensity corresponding to 80% of CV, with the animals running at 20 m/min (BLC = 3.90 ± 0.03 mmol/L). Therefore, the non-exhaustive protocol underestimate in 20% the MLSS.

Similar results for the exercise intensity corresponding to MLSS and BLC stabilization were obtained in earlier studies[9,10,22,23]. Manchado *et al.*[9] submitted sedentary rats to continuously running exercise at 10, 15, 20, 25 and 30 m/min. The MLSS was obtained at BLC of 3.9±1.1 mmol/L and at 20 m/min velocity. The value of maximal exercise intensity associated to aerobic condition observed in the present study and in Manchado *et al.*[9] was lower than the speed observed by Pillis *et al.*[8] (25 m/min), obtained by a different protocol. These authors determined the aerobic threshold in running rats using an incremental multistage treadmill exercise. The anaerobic threshold (AT) was estimated from individual plots of blood lactate vs treadmill speed and AT was considered the level of exercise intensity at which blood lactate concentration started to increase rapidly. Langfort *et al.*[15] also reported the same intensity for AT calculated as the speed of running corresponding to the individual breaking point of the lactate curve using the two-segment linear regression of sedentary rats (25 m/min).

These differences in blood lactate concentration at stabilization and MLSS intensity among the studies suggest the protocol-dependence of the blood lactate responses, as observed in different exercise tests for human subjects[24,25] and animal's protocols[4,10,23,26].

The non-exhaustive exercise test seems to be the interesting method for the evaluation of both human and animal model of endocrine-metabolic diseases, in which physical condition is severely compromised.

In summary, the results suggest that the non-exhaustive protocol used seems to be valid for the aerobic evaluation in sedentary running rats, but this protocol underestimates the MLSS in 20%. However, small adjustments are required in aerobic training velocity.

Acknowledgement

The study was supported by "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP – Proc. 04/07070-5). Grateful acknowledgment to Clarice Yoshiko Sibuya, Eduardo Custódio and José Roberto Rodrigues da Silva, for help.

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