Maximal lactate steady state in rats submitted to swimming exercise

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Abstract

The higher concentration during exercise at which lactate entry in blood equals its removal is known as ‘maximal lactate steady state’ (MLSS) and is considered an important indicator of endurance exercise capacity. The aim of the present study was to determine MLSS in rats during swimming exercise. Adult male Wistar rats, which were adapted to water for 3 weeks, were used. After this, the animals were separated at random into groups and submitted once a week to swimming sessions of 20 min, supporting loads of 5, 6, 7, 8, 9 or 10% of body wt. for 6 consecutive weeks. Blood lactate was determined every 5 min to find the MLSS. Sedentary animals presented MLSS with overloads of 5 and 6% at 5.5 mmol/l blood lactate. There was a significant increase in blood lactate with the other loads. In another set of experiments, rats of the same strain, sex and age were submitted daily to 60 min of swimming with an 8% body wt. overload, 5 days/week, for 9 weeks. The rats were then submitted to a swimming session of 20 min with an 8% body wt. overload and blood lactate was determined before the beginning of the session and after 10 and 20 min of exercise. Sedentary rats submitted to the same acute exercise protocol were used as a control. Physical training did not alter the MLSS value but shifted it to a higher exercise intensity (8% body wt. overload). Taken together these results indicate that MLSS measured in rats in the conditions of the present study was reproducible and seemed to be independent of the physical condition of the animals. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Wasserman and McIlroy (1964) first defined the term anaerobic threshold, in the sense that during exercise the abrupt increase of blood CO2 reflects a metabolic shift towards the anaerobic system. Kindermann et al. (1979), after accomplishment of incremental exercise tests in well trained athletes, postulated the occurrence of a metabolic transition between aerobic and anaerobic systems at blood lactate concentrations between 2.0 and 4.0 mmol/l, with a marked anaerobic threshold at 4.0 mmol/l. These data were in good agreement with the previous statement that the anaerobic threshold corresponds to a disproportional increase in blood lactate concentration in response to an exercise load (Mader et al., 1976). Later, Sjödin and Jacobs (1981)
considered the blood lactate level of 4.0 mmol/l as being the ‘onset of blood lactate accumulation’. In this sense during an incremental exercise test, with evaluations of blood lactate and exercise load, it has been postulated that the load corresponding to blood lactate levels of 4.0 mmol/l is the submaximal aerobic performance ($V_{OBLA}$), and that it can be calculated through the blood lactate–exercise intensity curve linear regression analysis.

In 1985, Heck and his colleagues performed constant load exercise tests in humans and evaluated blood lactate concentrations along the exercise session. The authors observed that independently of the individual aerobic capacity, the average ‘maximal lactate steady state’ (MLSS) value was achieved at 4.0 mmol/l. Thus, the production/removal of lactate in humans stabilized at a maximal blood concentration of 4.0 mmol/l. Since then, maximal lactate steady state became a common parameter used in the determination of the anaerobic threshold (Heck et al., 1985; Mader and Heck, 1986; Baldari and Guidetti, 2000). Although many attempts were made, the physiological basis for blood lactate accumulation during exercise has not yet been fully explained. Many studies on rates of lactate production, release and removal, among other parameters of lactate flux during exercise were reviewed along the last decade (Katz and Sahlin, 1990; Brooks, 2000). In these studies the assumption that the lactate formation during submaximal exercise is due to tissue hypoxia has been questioned and the ‘lactate shuttle’ and later, the ‘intra and extracellular lactate shuttles’ theories were introduced (Brooks, 2000). In light of these new theories, the existing data on lactate flux during submaximal exercise can be interpreted in alternative ways, considering that lactate is produced all the time in fully oxygenated cells and tissues and that lactate production, distribution and removal play equally important roles in blood lactate concentration (Katz and Sahlin, 1990; Brooks, 2000). Also, in recent years the literature provided evidences that the MLSS not always indicates the ‘anaerobic threshold’ and, sometimes, is independent of performance (Beneke, 1995; Beneke et al., 2000). Despite all this, the ‘anaerobic threshold’ and the MLSS determinations are still found useful in the assessment of subject’s endurance capacity (Palmer et al., 1999; Baldari and Guidetti, 2000; Jones and Carter, 2000; Billat, 2000).

For obvious reasons, a significant number of researches involving exercise have been conducted in laboratory animals, mainly rats, and blood lactate concentration was used in many of them for the determination of effort intensity. However, such a procedure is hindered by the lack of information about the lactate kinetics in rats. Since there are metabolic differences between humans and rats, it is reasonable to speculate on potential specie differences with regard to lactate flux during exercise. Even if the same general principles that regulate lactate flux are valid for both species, there may be quantitative differences in some parameters between them. In the case of treadmill-run for rats, the exercise intensity is elevated by increasing the speed, which enabled the determination of the anaerobic threshold in incremental treadmill exercise (Pillis et al., 1993; Langfort et al., 1996). In swimming exercise protocols for rats, incremental exercise is obtained by adding loads progressively heavier in relation to the body weight, attached to the animals chest or tail (Gobatto et al., 1991). The use of swimming rats as a model of exercise presents advantages over treadmill running, since swimming is a natural ability of the rat. This avoids the selection of the animals, which is necessary in experimental protocols using treadmill running. The major limitation of the swimming studies is the ignorance of the intensity of effort performed by the rat. In humans, it was reported that the anaerobic threshold may be shifted, depending on the exercise mode testing (Schneider et al., 2000). The literature lacks information about lactate flux in rats during swimming exercise. This impairs an objective standardization of swimming training protocols, since it is not known if there are effects of exercise mode on anaerobic threshold or maximal lactate steady state in rats.

The present study was designed to determine the maximal lactate steady state of sedentary rats submitted to swimming exercise supporting loads varying from 5 to 10% of the body wt., tied to the chest, and to verify if the lactate concentration at maximal steady-state remains unaltered after physical training.

2. Methods

2.1. Animals

All experiments involving the animals were
conducted in conformance with the policy statement of the American College of Sports Medicine on Research with experimental animals. Male Wistar rats, 90 days old, weighing 250–350 g at the beginning of the experiment and 400–435 g at the end, were used. During the whole experimental period, the animals received commercial chow for rodents and water ad libitum. The animals were housed in collective cages (4 rats per cage), in a room with light on from 06.00 to 18.00 h, at 25°C.

2.2. Adaptation to the water

All the rats were adapted to the water before the beginning of the experiment. The adaptation consisted of keeping the animals in shallow water at 31 ± 1°C (Harri and Kuusela, 1986), 5 days/week, from 09.00 to 21.30 h. The adaptation to the water proceeded along the entire experimental period. The purpose of the adaptation was reducing the stress without, however, promoting physical training adaptations.

2.3. Acute exercise-test protocol

Rats were submitted to swimming exercise supporting constant loads (lead fish sinkers, added to the chest) of 5, 6, 7, 8, 9 and 10% of body wt., in two tanks of 100 × 80 × 80 cm, subdivided into four compartments each, filled with water at 31 ± 1°C. This device enabled the evaluation of eight animals swimming alone at the same time. Each animal participated in six experimental tests with 1-week intervals between them, for 6 consecutive weeks. The sequence of the loads was distributed at random, and the same load was never used twice by the same animal. Each test consisted of continuous swimming for 20 min with one load. Blood samples for lactate determinations were collected five times: before the beginning and at each 5 min of exercise.

2.4. Exercise training

In another set of experiments, rats of the same strain, sex and weight, were trained to swim 60 min/day, 5 days a week, with an overload of 8% body wt. during 9 weeks, in the same device where the first series of experiments took place. Exercise sessions lasted 10 min on the first day of the training period and were increased by 10 min each 7 days. At the end of the 7th day the animals swam continuously for 20 min and at the end of the 14th day, they swam for 40 min. Continuous exercise (60 min) was performed from the 21st day until the end of the training period. Sedentary rats placed in shallow water at 31 ± 1°C, 30 min, 5 days/week, were used as controls. At the end of the training period and 48 h after the last exercise bout, all rats were submitted individually to two 20-min exercise test sessions: one without (0%) load and another supporting an 8% body wt. overload, with blood collection before the beginning and after 10 and 20 min of exercise for lactate measurement. There was a 3-day interval between the two tests.

2.5. 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 50 µl sodium fluoride (1%). To avoid blood lactate dilution with residual water at the tail or the animal, the rats were quickly dried with a towel immediately before blood collection. The lactate concentrations were determined in a lactate analyzer (YSI model 1500 SPORT).

2.6. Statistical analysis

The statistical procedure consisted of one-way ANOVA. When necessary, the Newman–Keuls post-hoc comparison test was used (Dawson-Saunders and Trapp, 1994). In all cases, the statistical significance was set at \( P < 0.05 \).

3. Results

The animals presented maximal lactate steady-state at 5.5 mmol/l blood lactate with an overload of 5 and 6% of body wt. There was a progressive increase in blood lactate concentration with the other loads (Fig. 1).

Exercise-trained rats maintained the maximal lactate steady-state at 5.5 mmol/l blood lactate but this condition was achieved with an overload of 8% of body wt. However, sedentary rats adapted to water, did not show blood lactate stabilization supporting the same (8%) overload. Sedentary and trained rats when submitted to an exercise session without overload showed initially a sig-
Fig. 1. Blood lactate concentrations during acute exercise tests supporting overloads corresponding to 5% (n = 12), 6% (n = 27), 7% (n = 26), 8% (n = 26), 9% (n = 25) and 10% (n = 27) of body wt. Results are mean ± S.E.M. In each panel, significant differences (P < 0.05) are: (a) vs. rest values; (b) vs. rest and 5 min exercise values; (c) vs. rest and 5 and 10 min exercise values; (d) vs. rest and all other exercise time values.

Fig. 2. Blood lactate concentrations of swimming sedentary (n = 12) and trained (n = 8) rats during exercise test sessions without (0%) overload or supporting an overload corresponding to 8% of body wt. Results are mean ± S.E.M. In each panel, significant differences (P < 0.05) are: (a) vs. rest values; (b) vs. rest and 10 min exercise values.
nificant increase and later a significant decrease in blood lactate (Fig. 2).

4. Discussion

In spite of the high correlation between muscle and blood lactate, it is a mistake to consider blood lactate concentrations only as a result of lactate release, since there are several pathways for blood lactate removal. The liver seems to play an important role, using lactate as a substrate for glucose production by means of gluconeogenesis (Ryan et al., 1993; Brooks, 2000). The heart is another organ that contributes to blood lactate removal since it uses lactate as an energy source (Bonen, 2000), but skeletal muscle itself seems to play the major role in lactate removal during and after exercise (Brooks, 2000; Donovan and Pagliassotti, 2000; Gladden, 2000).

The maximal lactate steady state represents the higher blood lactate concentration at which lactate removal capacity compensates its entry (Mader and Heck, 1986; Poole et al., 1988) and is considered an indicator of the endurance exercise capacity (Jones and Carter, 2000). In this sense, our data show that sedentary rats are able to keep a stable blood entry/removal ratio in workloads up to 6% of body wt. In higher loads, blood lactate concentrations increased with time, indicating higher production in relation to removal. This suggests that the lactate entry/removal ratio in sedentary male adult Wistar rats, submitted to acute swimming, reaches its maximal in workloads of up to 6%. Such information allows us to postulate that, for animals with such characteristics, loads below 6% of the body wt. can be considered ‘sub-threshold’. Considering the assumption mentioned above, the use of these ‘sub-threshold’ loads in the beginning of the physical training protocol is indicated, if the goal is an improvement of endurance exercise capacity.

The major contribution of the present study, however, is the demonstration that maximal lactate steady state for sedentary rats submitted to acute swimming exercise occurs at blood lactate concentrations of 5.5 mmol/l. In a previous study, Pillis et al. (1993) found an anaerobic threshold at blood lactate concentration of approximately 4.0 mmol/l in rats during a discontinued multistage treadmill exercise test and suggested that this finding might offer a potential application of blood lactate threshold for serial evaluations of animal working ability under various experimental conditions. This statement must be seen with care, since the results obtained in the present work suggest that the blood lactate threshold for rats may be shifted depending on the exercise-testing mode. This hypothesis is strengthened by the fact that Schneider et al. (2000) reported that, in humans, the threshold for blood lactate shifted between exercise test modes. Blood lactate threshold was found by the authors to be significantly lower for arm than for leg exercise.

In another set of experiments, we performed tests using sedentary and trained rats supporting an 8% body wt. load. The use of different load intensities is common in swimming protocols for rats. Mostly, loads of 5% (Pereira et al., 1994; Tassi et al., 1998; Galdino et al., 2000) and 8% (Azevedo, 1994) of body wt. are used. In the present study we chose the 8% load for training the rats based on the results described above, showing that loads up to 6% body wt. are sub-threshold in swimming protocols for untrained rats. As we intended to submit the rats to a long exercise-training period (9 weeks), we supposed that a 5% body wt. load, along this period, would become insufficient to promote any physical training adaptation in the animals. Considering this hypothesis, we submitted the rats during the first 21 days of the training period to short exercise sessions (10–40 min of exercise) supporting the 8% body wt. load and from the 21st day on, to 60 min of a continuous exercise session per day with the same load. While sedentary, rats did not show blood lactate stabilization, the trained ones achieved it at the concentration pointed previously as the maximal blood lactate steady state (5.5 mmol/l). These data suggested that the endurance capacity of the animals was improved (8% load instead of 5–6%), but the blood lactate concentration at which maximal lactate steady state was reached remained the same.

In light of the present data, we can say that training rats with an 8% body wt. load is inadequate and intense at the beginning of the process. However, this exercise intensity promotes an increase of the anaerobic threshold along the training period, what probably explains the stabilization of the blood lactate concentration in the exercise-trained rats. The stabilization of blood lactate with loads of 8% of body wt. in trained animals is probably due to muscle aerobic adapta-
tions leading to lower lactate production for the same relative and absolute workload (Jones and Carter, 2000) and/or to increased blood lactate removal (Gladden, 2000; Donovan and Pagliassotti, 2000). Furthermore, our results indicate that, in rats, as described by Heck et al. (1985), in humans, the maximal lactate steady-state seems to be independent of the aerobic capacity.

In spite of this, we can speculate that the use of loads higher than 6% of the body wt., in long lasting exercise sessions, at the beginning of a training period, could lead to undesirable effects of intense physical exercise, such as muscle lesions and incomplete substrate levels restoration between one exercise-session and the next. These effects can generate conflicting results and inconsistent experimental conclusions.

In summary, untrained rats performing swimming exercise supporting loads of 5–6% of body wt. allow a maximal blood lactate stabilization at 5.5 mmol/l. Physical training did not alter this value but shifted it to a higher exercise intensity (8% of body wt. load). Taken together, these findings indicate that the MLSS measured in rats in the conditions of the present study is fairly reproducible and seemed to be independent of the physical condition of the animals.

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