

BLOOD LACTATE RESPONSES TO HIGH-INTENSITY INTERMITTENT TRAINING IN RATS

EXERCISE AND
SPORTS SCIENCES



ORIGINAL ARTICLE

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ABSTRACT

During high-intensity intermittent muscle contractions for short periods of time there is an important involvement of glycolytic metabolism and consequent increased blood lactate concentrations. This study aimed to evaluate the blood lactate responses in Wistar rats submitted to high-intensity intermittent training (jump squat) protocol during 6 weeks, 3 sessions, 12 x/session, 60s of interval between sessions. There was significant increase of blood lactate concentrations during the acute bout of high-intensity intermittent exercise (basal blood lactate vs blood lactate after last effort, $P < 0.001$); however, after six weeks of training, there was significant reduction (49%) in blood lactate response to the exercise in comparison to the first session, $P = 0.0002$. The high-intensity intermittent exercise performed at intervals of 60 seconds stimulated the glycolytic system; nevertheless, the training promoted reduction in blood lactate responses to high-intensity intermittent protocol, suggesting hence improvement in phosphocreatine recovery capacity and in mitochondrial biogenesis.

Keywords: resistance training, blood lactate, glycolytic system.

INTRODUCTION

In animals models, the aerobic training protocols have been frequently used on treadmill¹ and swimming². Concerning the anaerobic training protocols, many studies have used efforts with jumps performed in water^{3,4} and on platform through electric stimulation^{5,6} in different nutritional⁷ and pathological conditions³. The training protocols with jumps are composed of 3-4 sets of 10-12 jumps with intervals of 60-90s^{6,7}. Additionally, the intensity increase is performed with weight addition corresponding to the body weight⁴ or a maximum repetition⁸.

High-intensity physical training performed for short periods of time has significant effects on the anaerobic glycolytic system, in a way that the increase of the ATP production is followed by increase of muscular lactate production⁹.

The use of blood lactate concentrations has been an instrument constantly used as a marker of exercise intensity in humans⁹ and in animal models². In addition to that, this metabolite has been recently used to indirectly estimate the contribution of the lactic anaerobic metabolism in the energetic supply¹⁰ as well as evaluation of the alactic anaerobic metabolism¹¹.

However, opposed to the aerobic training models, which are often characterized through the lactacidemic response and tests of aerobic capacity, the availability of studies about the lactacidemic response and consequently the participation of the glycolytic system and the consequent adaptations to this condition in exercises, which predominantly use the glycolytic metabolism for energy, are limited. Thus the aim of the present study was to investigate the lactacidemic responses and the participation of the glycolytic system during acute and chronic training of Wistar rats submitted to a *jump squat* high-intensity intermittent training model.

MATERIAL AND METHODS

Animals

Ten Wistar male adult rats (120 days) were kept in the experimentation animal facility in the origin university, in collective cages, not exceeding four animals per cage, in light/dark cycle of 12/12 hours and controlled temperature of $22 \pm 2^\circ\text{C}$. The animals were fed with standard food (Supra Lab – Alisul Ind. Alimentos LTDA., São Leopoldo, RS) and received water *ad libitum*. The experimental procedures used in the present study were approved by the Ethics in Animal Experimentation Committee of UNESP – Presidente Prudente Campus, law suit # 15/2009.

Training program

The high-intensity intermittent training was performed following the strength model adapted by Tamaki *et al.* (1992). The apparatus was designed so that the animal was immobilized on a metallic platform through an adapted vest attached to the animal's thorax (figure 1). In order to make the rat jump (complete knee and ankle flexo extension movement) lifting a load positioned on the posterior part of the vest, electric stimulation was applied using a metallic clip which involved the animal's tail tip connected to an electro stimulator, model Dualpex 961 by Quark, calibrated by Inmetro (National Metrology Institute, Industry Normalization and Quality)⁶. The used parameters were: frequency 1Hz, duration of 0.3s with 2s interval between each electric stimulation, and intensity was adjusted so that the animal performed the movement, ranging from 3 to 6mA. These parameters were adopted for being bidirectional pulses of null mean, not presenting electrolytic effects, allowing long duration applications with no risk of tissue injury⁵.

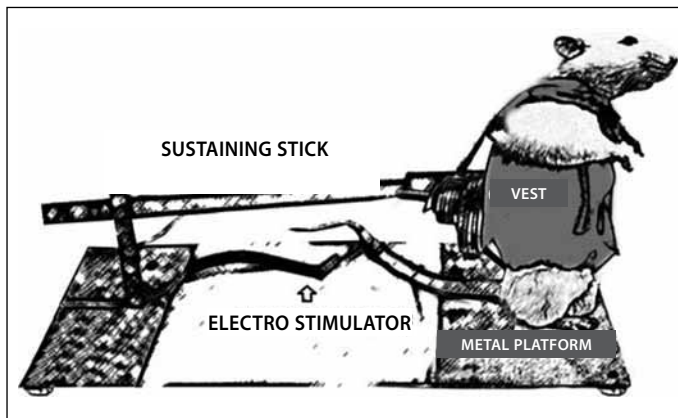


Figure 1. Model of the apparatus used for performance of jumping training (jump squat).

Experimental outlining

The animals were adapted to the exercise model one week before the beginning of the high-intensity intermittent training. The adaptation consisted in the performance of three sessions with no load increase composed of one, two and three sets of 12 repetitions from the first to the third day, respectively, with 24-hour interval between each session.

Subsequently, they were submitted to six weeks of high-intensity intermittent training. On the two first weeks the training was performed with no load increase and from the third week equivalent load to 50% of body weight (BW) was added, which was weekly monitored.

the training protocol consisted of three sessions a week which were performed in 24 hours intervals among them. each session consisted of three sets of 12 repetitions and 60s of intervals among the sets.

Blood collection and analysis

At every 15 days (six sessions), the lactacidemic responses were monitored during and at the end of the training. The blood samples were collected immediately after the first ($[La]_{1st}$), second ($[La]_{2nd}$) and third ($[La]_{3rd}$) sets and at the third (3rd min), fifth (5th min) and seventh (7th min) minute after the last jump set, and the highest lactacidemic value obtained at the end of the stimuli was taken as lactate peak concentration ($[La]_{peak}$).

The blood samples (25 μ L) were collected by puncture on the tale in a heparinized capillary and were immediately transferred to 1,5 mL Eppendorf tubes containing 50 μ L of NaF solution at 1%. The homogenized was later frozen for subsequent analysis in Yellow Springs electro enzymatic lactimeter, model 1500 Sport (figure 2).

STATISTICAL ANALYSIS

Data normality was confirmed with Shapiro-Wilk test. Body weight values and lactacidemic responses during the acute physical exercise session were compared and during six weeks of high-intensity intermittent training the one-way ANOVA for repeated measures was used, followed by Tukey *post hoc* test whenever necessary. In all cases the significance level was set at $P < 0.05$.

RESULTS

Significant alterations in the BW of the animals were not observed during the training period with weight ranging in about 400.88 ± 13.93 g ($n = 10$ animals). During the acute session, significant increase of 29%, 86% and 140% in $[La]_{1st}$, $[La]_{2nd}$ and $[La]_{3rd}$, respectively, concerning rest was observed. Moreover, the $[La]_{peak}$ was significantly higher than the values observed in rest and $[La]_{1st}$, $[La]_{2nd}$ and $[La]_{3rd}$ (figure 3). However, significant differences were not observed between the lactacidemic values observed on the third, fifth and seventh minute. Mean significant reduction of 32% in $[La]_{1st}$, 46% in $[La]_{2nd}$ and 48% $[La]_{3rd}$ in T2, T3 and T4 was observed when compared with T1 ($P = 0.0002$). The $[La]_{1st}$ in T3 and T4 was 24% and 25%, respectively, lower concerning $[La]_{1st}$ in T2 ($P < 0.05$). Additionally, the $[La]_{2nd}$ in T4 was significantly lower (25%) compared with T3 ($P < 0.05$). Nevertheless, the $[La]_{3rd}$ presented significant difference in T2, T3 and T4 only when compared with T1 (figure 4).

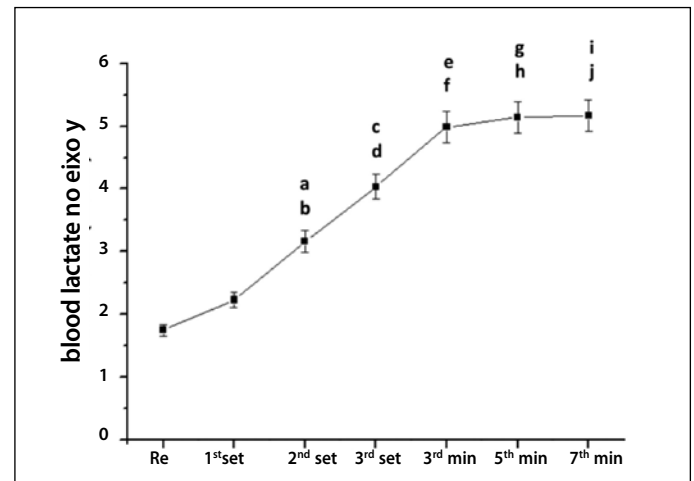


Figure 3. Mean values \pm standard error of the blood lactate concentrations obtained at rest (Re), immediately after the first, second and third sets of 12 jumps and after the third, fifth and seventh minute of recovery (3rd, 5th and 7th min, respectively). ^a $P < 0.001$ vs. Re; ^b $P < 0.05$ vs. 1st set; ^c $P < 0.001$ vs. Re and 1st set; ^d $P < 0.05$ vs. 2nd set; ^e $P < 0.001$ vs. Re, 1st set and 2nd set; ^f $P < 0.05$ vs. 3rd set; ^g $P < 0.001$ vs. Re, 1st and 2nd sets; ^h $P < 0.05$ vs. 3rd set; ⁱ $P < 0.001$ vs. Re, 1st and 2nd sets; ^j $P < 0.05$ vs. 3rd set.

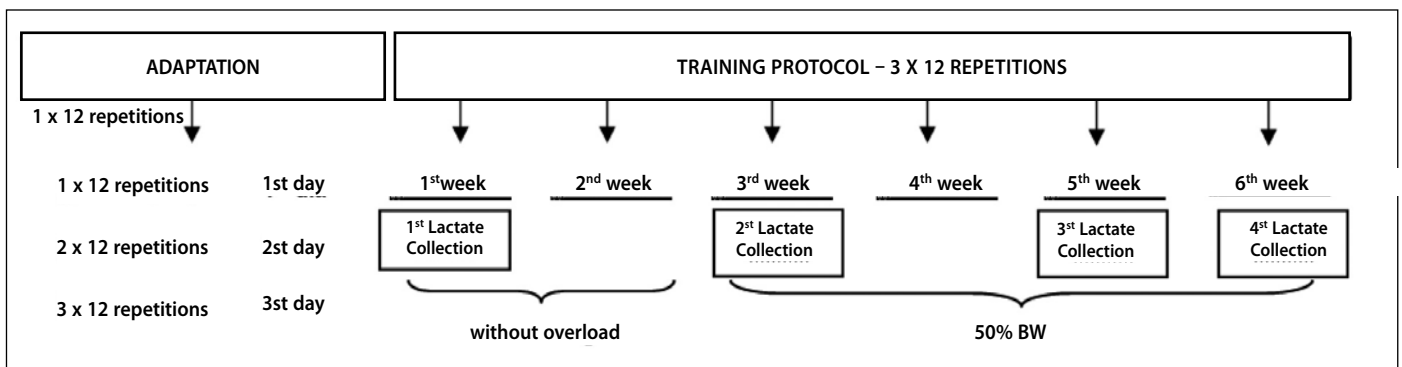


Figure 2. Training and collection for lactacidemia measures protocol.

The $[La^-]_{peak}$ obtained in T2, T3 and T4 presented reduction of approximately 35%, 39% and 49%, respectively, compared with the one obtained in T1 ($P = 0.0002$). Moreover, the $[La^-]_{peak}$ in T4 presented 22% of reduction compared with T2 ($P = 0.004$) (figure 5).

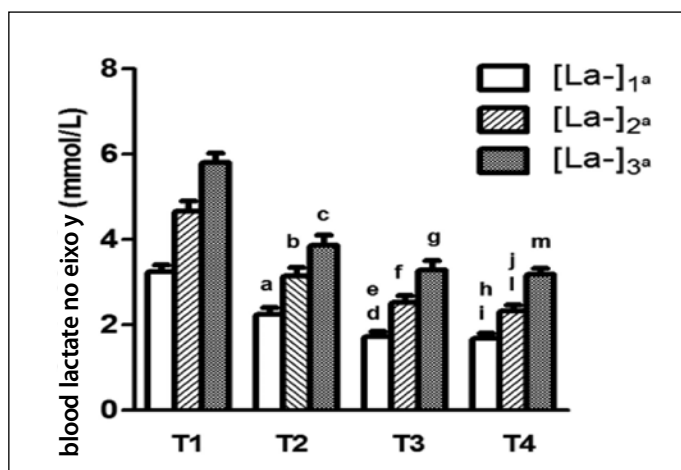


Figure 4. Mean values \pm standard error of blood lactate concentrations $[La^-]_{1st}$, $[La^-]_{2nd}$ and $[La^-]_{3rd}$ of the high-intensity intermittent training measured on the first session (T1), after 15 (T2), 30 (T3) and 45 (T4) days of training. ^a $P = 0.0002$ vs. $[La^-]_{1st}$ (T1); ^b $P = 0.0002$ $[La^-]_{2nd}$ (T1); ^c $P = 0.0002$ $[La^-]_{3rd}$ (T1); ^d $P = 0.0002$ vs. $[La^-]_{1st}$ (T1); ^e $P = 0.004$ vs. $[La^-]_{1st}$ (T2); ^f $P = 0.0002$ vs. $[La^-]_{2nd}$ (T1); ^g $P = 0.0002$ vs. $[La^-]_{3rd}$ (T1); ^h $P = 0.0002$ vs. $[La^-]_{1st}$ (T1); ⁱ $P = 0.03$ vs. $[La^-]_{1st}$ (T2); ^j $P = 0.0002$ $[La^-]_{2nd}$ (T1); ^k $P = 0.02$ $[La^-]_{2nd}$ (T2); ^m $P = 0.0002$ vs. $[La^-]_{3rd}$ (T1).

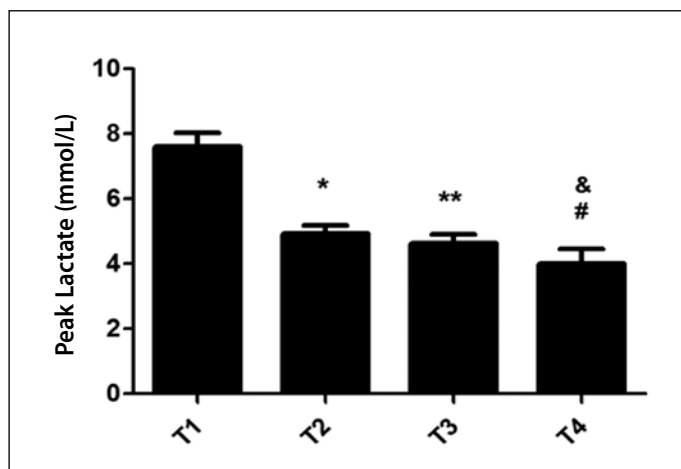


Figure 5. Values of $[La^-]_{peak}$ measured on the first session (T1), after 15 (T2), 30 (T3) and 45 (T4) days of high-intensity intermittent training. Values expressed in millimoles per liter (mmol/L) as mean \pm MSE.

DISCUSSION

Physical activity practice represents an important instrument for reversal of metabolic alterations of many diseases such as obesity, type 2 diabetes mellitus and cardiorespiratory diseases¹².

The blood lactate concentrations let us evaluate the predominance of the participation of the aerobic or anaerobic system in response to an acute⁹ or chronic physical exercise¹³. Therefore, they have been used for prediction of the physical exercise intensity with the goal to optimize performance and/or minimize the pathological metabolic alterations as well as to evaluate the metabolic responses to training¹⁴.

During the high-intensity exercise initial phases, the ATP is degraded through the ATPase myosin enzyme, while the phosphocreatine is degraded by the creatine kinase enzyme for the ATP resynthesis. In order to perform intense exercise longer than 12-15

seconds and shorter than three minutes of duration, the body mainly depends on the anaerobic metabolism for energy production¹⁴. When a high-intensity physical training is performed in short time periods, the increase of ATP production is followed by increase of the muscular lactate production⁹.

In the present study, during the high-intensity intermittent training session, it can be observed that the lactacidemic response presented progressive increase during the time, with peak lactate concentrations at the seventh recovery minute (figure 3). These observations demonstrate that the time interval adopted between each set (60 seconds) was not sufficient to cause complete replacement of the phosphocreatine (PCr) supplies, so the glycolytic system effectively participated in the ATP production during the high-intensity intermittent training session.

Increase in the blood lactate concentration was also observed by Gorostiaga *et al.*¹⁵ associated with the lower PCr contribution after 10 repetition maximum (RM) of knee extension (*leg press*) when compared with a 5RM set. Still in this study, the exercise protocol produced fatigue and alteration in the muscular PCr content, lactate and glycolytic intermediaries in higher levels¹⁵.

The higher values of blood lactate concentrations $[La^-]_{1st}$, 2nd $[La^-]_{2nd}$ and 3rd $[La^-]_{3rd}$ and $[La^-]_{peak}$ were observed on the first day of collection; other words, in the first training session (figure 4). It is known that the adrenaline and noradrenaline plasma concentrations increase during physical exercise¹⁶ and strong correlation between the catecholamine and lactate plasma concentrations has been reported¹⁷.

It is probable that in the first training session of the present study, although the animals have been previously adapted to the experimental model, higher activation of the β -adrenergic system has occurred, which may have influenced on the higher lactacidemic response in this training session when compared with the subsequent sessions (figure 4).

Physical training may result in alterations in the ATP and PCr levels stored in the muscle. Strength training promotes increase of approximately 20% of ATP and PCr¹⁸. Larsen *et al.*¹⁹ verified that the constant PCr recovery after performance of maximal isometric voluntary contraction of the tibialis anterior and vastus lateralis muscles with reduction of 50% of the PCr basal values, in active individuals is higher than in sedentary individuals. Likewise, Yoshida²⁰ observed higher recovery velocity of PCr (63%) in the biceps femoris muscle of young runners when compared with sedentary young subjects. This difference magnitude in the constant recovery of PCr was similar to the one found by Larsen *et al.*¹⁹.

Expressive decrease in the lactate peak concentration was observed on the last collection day of blood lactate when compared with the second day. This reduction of peak lactate concentration after six weeks of high-intensity intermittent training suggests improvement of the PCr resynthesis system; that is, increase of the muscular PCr supplies.

The PCr resynthesis rate is directly proportional to the oxidative phosphorylation rate and therefore, it reflects contributions both of glycolytic and oxidative metabolism^{21,22}. In agreement with this statement, Mogensen *et al.*²³ reported mitochondrial respiration rates and citrate synthase activity 41% higher in the vastus lateralis muscle of trained individuals when compared with untrained ones.

Forbes *et al.*²² also used the intervalled maximal exertions protocol and demonstrated improvement in the PCr resynthesis constant in the quadriceps of adults after two weeks of training.

Although improvement in oxidative capacity as a result of aerobic training is a consensus in the exercise physiology field, recent studies have suggested that exercises performed at critical intensities induce alterations in the oxidative capacity²⁴. In a study carried out in young individuals, six weeks of intervalled maximal efforts induced improvement in markers of oxidative capacity in the vastus lateralis muscle, similarly to in an aerobic training²⁵.

According to our results, it can be concluded that the reduction of the lactacidemic values in response to this training model was promoted by increase, especially of the PCr supplies (figure 5). However, considering that high-intensity efforts performed in a short period of time stimulate the mitochondrial biogenesis by increase in PGC1- α expression (peroxisome proliferator-activated receptor- γ coactivator 1 α), a transcriptional factor involved in the gene regulation of the cellular energetic metabolism, as proposed by Gibala²⁴, the low lactacidemic response in this model may also be a result from the improvement in the oxidative capacity.

Thus, this experimental model let us propose that high-intensity intermittent physical training may be used to promote adaptations in metabolism with simultaneous improvement of use of energetic substrate due to improvement in mitochondrial biogenesis.

This study let us conclude that in the experimental model of high-intensity intermittent physical exercise there is significant participation of the glycolytic system. However, if chronically performed, high-intensity intermittent training promotes alterations in the energetic metabolism which implies in reduction of the participation of this system in the ATP production.

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