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Responses of Blood Lactate Concentration in Aerobic and Anaerobic Training Protocols at Different Swimming Exercise Intensities in Rats

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ABSTRACT

Dos-Santos JW, de Mello MAR. Responses of Blood Lactate Concentration in Aerobic and Anaerobic Training Protocols at Different Swimming Exercise Intensities in Rats. **JEPonline** 2011;14(3):34-42. Recently, we published in **JEPonline**, 2010;13(5):27-43, a swimming periodized experimental training model in rats in which different training protocols (TP) were classified in aerobic (A) and anaerobic (AN) intensity levels. The purpose of the present study was to verify if the classification of the TP used in the periodized training experimental model presented the blood lactate concentration [La] response adequate to the aerobic and anaerobic intensities levels. Twenty three male Wistar rats were divided into three groups. Two groups of swimming training (continuous, CT, n = 7, and periodized training, PET, n = 7) rats were evaluated during 5 weeks in eight different TP (TP-1 to TP-8) through the analysis of the [La] response. The third group was the sedentary control (SC, n = 9). The TP were classified in five intensity levels, three aerobic (A-1, A-2, A-3) and two anaerobic (AN-1, AN-2). Analysis of variance (ANOVA one-way, P<0.05) indicated significant differences in the [La] among the TP and among the five intensity levels. All TP of the A-2 and A-3 intensity levels differed from the A-1 and AN-1. The A-1 and AN-1 also differed among them. These findings demonstrate that the TP were classified properly at different levels of aerobic and anaerobic intensities, as based on the [La] response in a way similar to that of high performance swimming with humans. The results offer new perspectives for the study of exercise training in swimming rats at different levels intensity for performance or for health.

Key Words: Exercise, Lactate, Level, Rat

INTRODUCTION

The swimming exercise in rats is an experimental model that is widely used for evaluating the effects of training in pathological and physiological conditions such as obesity (1,2), diabetes (4,9,13), pregnancy (3), malnutrition (7), and to verify the effects on glucose metabolism (15) and fatty acid oxidation (16). Aerobic continuous training is the most commonly used swim training exercise. However, load different training, as expressed in percentage of body mass (BM) is also common, especially 0 and 2 % BM (15,16), 2% BM (9) and 5% BM (1,2,4,5,7,15). Intensive interval training, with anaerobic characteristics, has also been studied in the swimming exercise model in rats (1,15,16). However, despite several swimming exercise training protocols used in different experimental studies, the training intensities have not yet been quantify and applied appropriately in swimming exercise in rats. This is the case despite the assessment of aerobic capacity in humans that has been adapted for the swimming rats, maximal lactate steady state (MLSS) (8), anaerobic threshold through the lactate minimum test (17) and critical power model (11).

Recently, we published in **JEPonline** a periodized experimental training model for swimming exercise in rats (6). The model was adapted from high performance swimming training in humans, which combined different training protocols (e.g., continuous and interval training with aerobic and anaerobic characteristics). That study presented the main results of Dos-Santos's doctoral thesis (5) which had others objectives, among then to classify the training protocols at different intensity levels through the use of blood lactate concentration [La]. Since the [La] has become a common practice for performance evaluation and training control in competitive swimming in humans (10,14) and other sports, and its use in the evaluation of the effects of training in swimming rats (4, 5,15), the aim of this study is to verify if the training protocols used in the swimming periodized training experimental model in rats (6) presented the [La] responses adequately at different intensity levels of aerobic and anaerobic training protocols.

METHODS

Animals and Treatment

The animals were evaluated during the training period carried out by the Dos-Santos and de Mello's study (6). Male Wistar rats (n = 23), 90 days old, kept in cages (37x31x16 cm), 3 to 5 rats per cage, in a controlled room with 12 hr light/dark cycles at 22 ± 1 ºC with free access to water and food (Purina crow for rodents). The animals were separated into three groups. One group was composed of animals in the sedentary control group (SC, n = 9), which consisted of only spontaneous movement in the cages. Two groups performed swimming exercise training 5 days/week for five weeks: (a) continuous training group (CT, n = 7), and (b) a periodized experimental training (PET, n = 7). Body mass (BM) and food uptake were measured weekly. All the experimental procedures were carried out in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences and the guidelines of the Brazilian College of Animal Experimentation (COBEA) with the approval of the local Ethics Committee, UNESP- Univ Estadual Paulista (Process nº.: 1501/46/01/08).

Training

The training protocols were those used by Dos-Santos and de Mello (6). Briefly, there was a period of adaptation during a four-week period, which consisted of progressive training with the CT and PET groups until the rats could swim 40 min with 5% BM load. After four weeks, the animals of the CT and PET groups engaged in swimming training in their respective groups for five weeks. The CT group was trained only with continuous training, 5% BM, 40 min/day. In the PET group, the animals performed a combination of aerobic continuous training with aerobic and anaerobic interval training. The training is based on the classic periodization training model proposed by Matveyev (12) in order to obtain the "peak performance" at the end of the experiment (Table 1). Therefore, in PET, there were daily variations of the training methods during the five weeks. Both groups performed the swimming training in tanks with individual partitions (PVC tubes, 25 cm diameter), with water 45 cm deep. The daily training load was adequate in “mini bag” (tactel) with an adjustable elastic band fixed to the anterior part of animal’s thorax and a Velcro opening to add lead pieces.

Table 1. Details of periodized training (PET) with the daily training sessions in five weeks and the identification of training protocols (TP) in day which blood samples were collected.

Week	Specifications	Daily training sessions of PET group					
1	Week days	Mon	Tue	Wed	Thu	Fri	Sat-Sun
	Rep x time x rec	1x20xØ	1x30xØ	6x4x1.5	1x60xØ	4x6x0.5	-
	Load (% BM)	(2.5%)	(6.0%)	(8.0%)	(3.0%)	(6.5%)	
	Evaluated TP		**TP-2**		**TP-1**		-
2	Week days	Mon	Tue	Wed	Thu	Fri	Sat-Sun
	Rep x time x rec	1x35x (-)	6x4x1.5	6x0.5x3.0	6x2.5x1.0	6x6x0.5	-
	Load (% BM)	(6.0%)	(8.0%)	(50.0%)	(9.0%)	(6.5%)	
	Evaluated TP		**TP-5**				-
3	Week days	Mon	Tue	Wed	Thu	Fri	Sat-Sun
	Rep x time x rec	4x1.5x5.0	5x6x0.5	5x2.5x1.0	6x0.5x3.0	5x6x0.5	-
	Load (% BM)	(25.0%)	(8.0%)	(10.0%)	(50.0%)	(8.0%)	
	Evaluated TP						-
4	Week days	Mon	Tue	Wed	Thu	Fri	Sat-Sun
	Rep x time x rec	3x6x0.5	4x1.5x5.0	4x2.5x1.5	4x0.5x3.0	4x5x0.5	-
	Load (% BM)	(8.0%)	(25.0%)	(10.0%)	(50.0%)	(8.0%)	
	Evaluated TP		**TP-7**		**TP-8**		-
5	Week days	Mon	Tue	Wed	Thu-Fri	Sat	Sun
	Rep x time x rec	3x0.5x3.0	3x2.5x1.5	4x6x0.5	-	3x3x0.5	1x4xØ
	Load (% BM)	(45.0%)	(10.0%)	(8.0%)		(9.0%)	(8.0%)
	Evaluated TP		**TP-6**	**TP-4**	-		-

Adapted of Dos-Santos and de Mello (6). TP = training protocols; Rep = number of repetitions; time = time of exercise; Rec = recovery; % BM = body mass percent; Ø = without recovery (continuous training). Time of exercise and recovery were expressed in minutes. Note: TP-3 was evaluated in CT group.

Training Protocols Evaluated

Eight different training protocols (TP), TP-1 to TP-8, (refer to Table 2) were assessed and classified into five intensity levels, according to the demands of aerobic [(A), A-1, A-2 and A-3] and anaerobic [(AN), AN-1 and AN-2] metabolism. The training protocol TP-3 was tested with rats trained in the CT group, while the other protocols (TP-1, TP-2, TP-4, TP-5 to TP-8) were part of the periodization model performed in the PET group. Each training protocol was assessed only during one training session between the 5th and 9th week of the experiment (specific training period). Two to 4 blood samples (25 µL) were collected during each training session from a cut at the tip of the tail to determine the [La] responses. One blood sample was collected in rest from each rat. The blood samples were diluted in 50 µL of NaF (1%), frozen and subsequently analyzed. The lactate concentrations were determined by electrochemical method in lactate analyzer (YSL 1500 STAT, USA).

Table 2. Classification of the training protocols (TP-1 to TP-8) in aerobic (A-1, A-2 and A-3) and anaerobic (AN-1 and AN-2) intensity levels and the moment of blood samples collection.

Intensity Level	Training Protocols	Rep x time x rec	Load (% BM)	Moment of blood collection (min)
A-1	TP-1 (n = 24)	1 x 60 x Ø	3.5	15, 30, 45, 60
	TP-2 (n = 18)	1 x 30 x Ø	6.0	10, 20, 30
A-2	TP-3 (n = 24)	1 x 40 x Ø	5.0	10, 20, 30, 40
	TP-4 (n = 18)	4 x 6.0 x 0.5	8.0	6, 19, 25.5
A-3	TP-5 (n = 12)	6 x 4.0 x 1.5	8.0	9.5, 20.5
	TP-6 (n = 12)	3 x 2.5 x 1.5	10.0	2.5, 10.5
AN-1	TP-7 (n = 12)	4 x 1.5 x 5.0	25.0	(1.5, 6.5)§, (21, 26)§
AN-2	TP-8 (n = 12)	4 x 0.5 x 3.0	50.0	(0.5, 3)§, (14, 17)§

Number of blood samples (n) = number of rats X number of blood samples during each training protocol; Rep = repetitions; Rec = recovery; Ø = without recuperation (continuous training); § = lactate peak (the highest value between the beginning and the end of the recovery after one both: AN-1 between 0 and the 5th min e AN-2 between 0 and the 3th min). Time of exercise and recovery were expressed in minutes.

Statistical Analyses

Data presented normality by the Shapiro-Wilk test. A one-way Analysis of Variance (ANOVA) was used to evaluate the statistical significance. The values were expressed in mean ± SEM (note: the [La] values of each training protocol considered the number of all blood samples from each animal in each TP). The significance level was fixed in 5% ($P=0.05$). Group sample size resulted in statistical power value more than 0.96 to $n \geq 12$. All calculations were performed using Statistica 7.0 software.

RESULTS

Training protocols (TP) were classified at different levels of intensity, since the [La] responses were different among them (i.e., in the majority of the cases) in accordance to the aerobic and anaerobic intensity levels. The [La] response of all TP classified at A-2 and A-3 intensity level differed from TP-1 (A-1) and TP-7 (AN-1). TP-1 and TP-7 differed between then (Table 3). TP-5 differed from TP-6 (both A-3) and did not differ from TP-4 (A-2). The TP-8 differed from all the TP, except from TP-3 and TP-5. Considering the [La] mean total in each intensity level (A-1, A-2, A-3, AN-1 and AN-2), there were differences among them, except A-3 that was not different from AN-2. The [La] response at rest was similar among the experimental groups (Table 4), but different from of all exercise intensity levels, except for TP-1.

Table 3. Blood lactate concentration from training protocols (TP-1 to TP-8) and at aerobic (A-1, A-2 and A-3) and anaerobic (AN-1 and AN-2) intensity levels.

Training Protocols	Blood lactate (mmol·L^{-1})	Intensity Level	Blood lactate (mmol·L^{-1})
TP-1 (n = 24)	1.6 ± 0.1[b,c,d,e,f,g,h]	A-1 (n = 24)	1.6 ± 0.1[¦,§,¥,£]
TP-2 (n = 18)	3.3 ± 0.4[a,e,f,g,h]		
TP-3 (n = 24)	3.0 ± 0.3[a,e,f,g,h]	A-2 (n = 80)	3.6 ± 0.7[*,§,¥,£]
TP-4 (n = 18)	4.0 ± 0.4[a,f,g]		
TP-5 (n = 12)	4.9 ± 0.5[a,b,c,f,g]	A-3 (n = 24)	5.7 ± 0.3[*,¦,¥]
TP-6 (n = 12)	6.5 ± 0.4[a,b,c,d,e,g,h]		
TP-7 (n = 12)	9.0 ± 0.6[a,b,c,d,e,f,h]	AN-1 (n = 12)	9.0 ± 0.6[*,¦,§,£]
TP-8 (n = 12)	4.6 ± 0.4[a,b,c,f,g]	AN-2 (n = 12)	4.6 ± 0.4[*,¦,¥]

Mean ± SEM (n = number of blood samples X number of rats evaluated in each TP. Note: At A-2 and A-3 intensity level the "n" presented is the sum of respective TP). Significance difference (ANOVA one-way, P=0.05). Difference among TP (letters): a ? TP-1; b ? TP-2; c ? TP-3; d ? TP-4; e ? TP-5; f ? TP-6; g ? TP-7; h ? TP-8. Difference among intensity level (symbol): * ? A-1; [¦] ? A-2; [§] ? A-3; [¥] ? AN-1; [£] ? AN-2.

DISCUSSION

The purpose of this study was verified if [La] presented an adequate response at the different intensity levels of aerobic and anaerobic training protocols in accordance with the classification proposed in the 2010 **JEPonline** research (6). Training protocols, TP, (TP-1 to TP-8) were classified properly at aerobic (A-1; A-2; A-3) and anaerobic (AN-1 and AN-2) intensity levels. All TP of the A-2 and A-3 intensity levels differed from the TP-1 and TP-7, and both also differed among them. Only at the A-3 intensity level, the TP-5 presented a [La] response not expected, because it differed from TP-6 (A-3) and did not differ from TP-4 (A-2). The TP-8 differed from all the TP, except TP-3 and TP-5, but that is not a problem since TP-8 (AN-2) was proposed to develop the alactic metabolism. Also, according to Maglischo (10), the [La] is not a good indication to classify power exercise intensity. The dissociation between stimulus alactic and lactic is very difficult, that is, even if the training had been carried out in short duration and high intensity there will be some lactate production.

Table 4. Blood lactate concentration in rest.

Groups	Blood lactate (mmol·L^{-1})
SC (n=9)	1.4 ± 0.2
CT (n=7)	1.1 ± 0.1
PET (n=7)	1.3 ± 0.1
T tal (n=23)	1.3 ± 0.1

Mean ± SEM

Our results were in a similar way to that proposed for high performance swimming with humans. Considering the [La] total mean in each intensity level (A-1, A-2, A-3, AN-1 and AN-2), [La] was similar to the proposed by Maglischo (10) to classify intensity level in swimming human beings (A-1 = 1-3; A-2 = 3-5; A-3 = 4-6; AN-1 = 8 to 9 mmol·L^{-1} and AN-2 = no indication, because present power/velocity exercise). Since it differed among them, except at A-3 that was not different from AN-2 intensity level. Despite the differences between humans and rats species and the characteristics of swimming exercise, the similarity in [La] between our results and those proposed for swimming humans is an indication that the training protocols evaluated in this study can be applied to study of training intensity in swimming rats.

TP-1 was proposed as a light intensity level (3% BM) with a load ~50% lower than MLSS, lasting 60 min (A-1 intensity level) and [La] was significantly lower than all others TP, which characterizes this TP as a light aerobic exercise with the oxidative characteristic (1.6 $mmol{\cdot}L^{-1}$), lower than the MLSS (5.5 $mmol{\cdot}L^{-1}$) in swimming rats (8). Loads lower than 3% BM were used to verified effects of training in swimming rats. A training protocol with a load of 2% BM, 60 min daily, 5 times/week counteracted the symptoms of alloxan-diabetes rats (9). Other low intensity training protocol (2 x 3 hr, 45 min of recovery interval, with load combination of 0 to 2% BM) improved the content of Glut-4 and the transport of glucose (15) and the fatty acid oxidation enzyme activity (16) in the skeletal muscle, performed during only eight and 10 days, respectively. To our knowledge, the chronic effects of the TP-1 have not been verified in any study. However, considering the similarity between the load of TP-1 (3% BM) and the load used in other studies (9,15,16), (2% BM), it is possible that the TP-1 will present similar effects to the training protocols with load of 2% BM.

At the A-2 intensity level, two continuous training protocols were evaluated: TP-2 (6% BM load, lasting 30 min) and TP-3 (5% BM load, lasting 40 min), and one interval training protocol, TP-4 (4 x 6 min of exercise supporting a load of 8% BM). Those TP were properly classified in the same intensity level ([La] = 3.3, 3.0, and 4.0 $mmol{\cdot}L^{-1}$, respectively). Continuous training performed at 5% BM during 60 min presented a similar [La] to the TP of A-2, 3.4 $mmol{\cdot}L^{-1}$ (5). Combinations of duration (30 to 60 min) and load (5-6% BM) used in continuous training may provide similar [La] results as well as an interval training protocol with a lower time of duration (24 min, 4 x 6 min) and a higher load (8% BM), TP-4. It was not our purpose to compare the "effects of training" among specific protocols studied. However, the study that compared the efficacy of training protocols on physiology parameters can help to clarify our results. Two protocols performed with a similar load to the three A-2 protocols of the present study were compared among themselves (continuous training, 5% BM, 60 min of duration) and one interval training protocol (60 x 0.5 min at 10% BM with 0.5 min of recovery interval). Each presented similar effects on glucose metabolism by skeletal muscle in vitro (5). Considering the results together, we speculate that despite the effects of training protocols of A-2 intensity level not having been tested yet, it is possible that it can provide similar training effects to those already verified in the study that used similar training protocols to this study. However, the evaluation of training effects is a limitation of this study (which can be verified in futures studies).

The A-3 intensity level was tested only by interval training protocols and corresponded at the 50% above the A-2 intensity level. The training protocol of A-3 intensity level was designed with the purpose to simulate the training equivalent to maximal oxygen consumption (VO_2 max), as used in swimming with humans. The [La] response to the TP-5 protocol was significantly lower than the [La] for TP-6, yet was similar to TP-4 (A-2). The load employed in TP-5 (8% BM), the same in TP-4, combined with 1.5 min of recovery between bouts was not a good option to A-3 intensity level. The TP-5 was the TP that did not perform as expected. On the other hand, TP-6 characterized very well the A-3 intensity, since the [La] response of TP-6 was higher than all protocols of the A-2 intensity level. Perhaps, if the load of TP-5 had been increased from 8 to 9 or even10% BM and/or the number of repetitions increased from 6 to 8-10, then, the TP-5 would present [La] similar to TP-6 and higher than all the TP of A-2 intensity.

Based on the results from TP-5 and TP-6, we believed that the load to simulate equivalent intensity at VO_2 max in swimming rats is between 9 to 10% BM, performed in bouts of 3 to 4 min. Despite the TP-6 [La] response different from all A-2 TP yet similar to the [La] of intensity level (A-3) that corresponds to swimming in humans, the aerobic training protocol with load above anaerobic threshold with the intention to exercise the rats at VO_2 max must be seen with precaution. To our knowledge, the determination of VO_2 max in swimming exercise in rats has not been described in any study. Nonetheless, it is reasonable to conclude that A-3 intensity level was correct because the load is above anaerobic threshold, the rats supported a load lasting 2.5 to 4 min, [La] was significantly different of A-2 TP, lower than AN-1 and similar to the [La] of A-3 proposed by Maglischo (10) for swimming in humans. Interval training protocol with similar characteristics to the A-3 protocols (11 x 4.0 min at 7.5% BM and 1.5 min of recovery) that were carried out for 60 min presented [La] = 5.8 mmol·L^{-1} and induced higher lactate production and glycogen synthesis by the soleus muscle. Compared to the continuous training (60 min at 5% BM) (5), it indicates that interval training can present better physiological effects than continuous training using anaerobic metabolism.

The two anaerobic TP had different purposes. In the TP-7 (AN-1), the load was 25% BM and the bouts were performed for 1.5 min with a 5-min recovery interval, in order to stimulate the lactic (anaerobic) metabolism. It was achieved since the rats had a mean [La] response of 9.0 mmol·L^{-1}. Using a training protocol with a load lower than 25% BM (14% BM, 14 x 20 sec, 10 sec of recovery interval), the rats trained only 8 days (280 sec/days, i.e., 14 x 20 sec), the [La] response increased to 11 mmol·L^{-1} at the end of a exercise session and the content for glucose transporters (Glut-4) and glucose transport (2-deoxyglucose) presented similar responses in comparison to a long lasting training protocol of 6 hr of exercise (2 x 3 hr, 45 min of recovery interval and a load combination of 0 to 2% BM). Due to similarity among TP-7 and the other two training protocols, one described by Terada et al. (15), 14% BM, 14 x 20 s, 10 sec of recovery interval, and other described by Braga et al. (1), 15% BM, 90 x 15 s, 15 sec of recovery interval, the TP-7 presents similar training effects. However, this should be investigated in future studies.

TP evaluated at AN-2 intensity level (TP-8) was proposed to stimulate the anaerobic (alactic) metabolism. In TP-8, performed with 50% BM, 4 x 0.5 sec, 3 min of recovery interval, the [La] response (4.6 mmol·L^{-1}) did not differ only from TP-4 (A-2) and TP-5 (A-3). The intensive exercise and short duration also stimulates the anaerobic glycolysis and, therefore, induces lactate production. In fact, the [La] response is not a good indicator for the alactic anaerobic exercise. In high performance swimming training with human, Maglischo (10) considers it inappropriate to use [La] as an index for the anaerobic alactic intensity since power exercise (high load and short duration) is not sufficient to raise excessively the [La] as occurs in lactic anaerobic exercise. Certainly, the characterization of alactic and lactic anaerobic exercises is a limitation of the swimming exercise with rats, since with a heavy load (25 to 50% BM) the rats alter the pattern of movement because they cannot swim at water surface and to combine swimming with jumps. The numbers of jumps was counted during the training session of TP-7 and TP-8 and presented 38 ± 3 and 16 ± 1 jumps per set, respectively (6). A similar training protocol (50% BM, 10 x 30 sec, 1 min of recovery interval) to the TP-8 (50% BM, 4 x 30 sec, 3 min of recovery interval) was used in a study with rats that consumed a high-fat diet while presenting similar effects in comparison to the continuous training, 60 min with 5% BM (2). The training with high load (to stimulate anaerobic metabolism) can be seen as a limitation in the swimming training in rats. However, we do not see a problem in this procedure because to increase the performance, in human swimming training, dry-land strength training and in-water resisted training are carried out with auxiliary equipments and performed in places different from those where the specific training and the competitions occurs. If the aim of training exercise is to improve performance or health, the training methods could not be a limitation since the aims are achieved.

The [La] responses presented different results according the characteristics of TP (load, duration, and number of bouts). The [La] responses demonstrated that it is possible to classify TP in aerobic and anaerobic intensity levels. The combination of load, duration of exercise, and of recovery between bouts also demonstrated that different TP can present similar [La] within same intensity level, i.e., A-2 intensity level. Obviously, we do not know the effects of each TP isolated, because we did not test in isolation. This study verified only that the [La] responses during one training session in rats trained in a training protocol that combined several TP (i.e., periodized training).

CONCLUSIONS

The TP used by Dos-Santos and de Mello (6) were classified properly at different levels of aerobic (A-1, A-2 and A-3) and anaerobic (AN-1 and AN-2) intensities, based on [La] in a similar way to that of high performance swimming with humans. Despite some limitations in swimming exercise with rats, and in comparing to the swimming exercise in humans, the TP evaluated in this study (TP-1 to TP-8) open new perspectives for the study of exercise training in swimming rats at different levels intensity, both for athletic performance and for health.

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